Synaptic transmission from splanchnic nerves to the adrenal medulla of guinea-pigs

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1. Membrane potentials were recorded with conventional intracellular microelectrodes from chromaffin cells in isolated, bisected adrenal glands from guinea-pigs.

2. All cells were electrically excitable and responded to depolarizing current with all-or-nothing action potentials that were blocked by tetrodotoxin.

3. Input resistance was 180 ± 14 MΩ and this was lower than that reported for isolated chromaffin cells using patch electrodes.

4. All cells responded to transmural stimulation with action potentials that arose from excitatory synaptic potentials in response to the excitation of one or more preganglionic fibres, many having strong synaptic action. Other fibres had weaker synaptic action but in all cases, maximal transmural stimulation caused depolarization well above threshold for action potential initiation.

5. Spontaneous excitatory synaptic potentials were observed whose frequency was greatly increased by repetitive stimulation at 10 or 30 Hz.

6. No evidence was found for the desensitization of nicotinic receptors in response to acetylcholine released from presynaptic nerve terminals.

7. These experiments show that there are many similarities between the responses to splanchnic nerve stimulation of guinea-pig chromaffin cells *in situ* and sympathetic ganglion cells from the same species.

Nearly 20 years ago Viveros (1975) pointed out that 'there are no reports of direct recording of the intracellular potential (of adrenal chromaffin cells) during splanchnic nerve stimulation'. Since that time there have been many studies on the physiology, biochemistry and pharmacology of the adrenal medulla and on chromaffin cells isolated from it. Their ion channels have been described (Fenwick, Marty & Neher, 1982*a*, *b*; Marty & Neher, 1985). Much is known about their nicotinic and muscarinic receptors (Role & Perlman, 1983; Inoue & Kuriyama, 1990, 1991) as well as their receptors for other biologically active agents such as neuropeptides (Marley & Livett, 1985). Nevertheless, we are unaware of any reports of responses to stimulation of the preganglionic nerve supply to the adrenal medulla on the membrane potential of chromaffin cells other than a report by Iijima, Matsumoto & Kidokoro (1992). They used a voltage-sensitive dye and an array of photosensitive diodes to study synaptic transmission in slices from rat adrenal glands. Nerve stimulation caused the initiation of action potentials associated with prolonged synaptic potentials. They concluded that cells were arranged in clusters, each cluster receiving inputs from about four nerve fibres.

We have used conventional intracellular microelectrodes to record membrane potentials of chromaffin cells in bisected adrenal glands isolated from guinea-pigs. Transmural electrodes were used to stimulate the splanchnic nerve supply (Wakade, 1981). In the present study we describe our initial observations on the characteristics of these cells *in situ* and their responses to nerve stimulation. We have found that guinea-pig chromaffin cells show many similarities with certain sympathetic ganglion cells from the same species – not a surprising observation in view of their common embryological origin. A brief account of these results has been presented previously (Tonta, Holman, Coleman & Parkington, 1993).

METHODS

Young guinea-pigs of either sex (mainly female, 250–450 g) were killed by decapitation. Left adrenal glands were removed and placed in ice-cold physiological saline solution in a chilled dissecting dish. Glands were partially bisected leaving the hilum intact and then pinned out, butterfly fashion, onto the silicone base of the recording chamber (Model QL-2 with temperature control unit TCU-2, Fine Science Tools, North Vancouver, BC, Canada) through which flowed physiological

saline solution containing (mM): NaCl, 120; KCl, 5; $CaCl_2$, 2·5; KH_2PO_4, 1; $MgSO_4$, 1·2; $NaHCO_3$, 25; glucose, 11; gassed with 95% O_2–5% CO_2.

The meniscus of the perfusing solution was adjusted to just above the highest point of the preparation. The bath (3 ml capacity) was continuously perfused at a rate of 2 ml min^{-1} and the temperature was maintained at 29–31 °C. The preparation was visualized with a binocular dissecting microscope. The transmural stimulating electrodes consisted of two silver wires that were coated with $AgCl_2$. One was permanently attached to the base of the chamber and the second was lowered onto the surface of the preparation. Stimuli were delivered through an isolation unit (Grass SIU5, Quincy, MA, USA) at frequencies of < 0·3 Hz unless otherwise stated (see Results). Pulse width was < 0·3 ms.

Membrane potentials were recorded using conventional intracellular glass microelectrodes (P-87 electrode puller, Sutter Instruments, Novato, CA, USA) filled with 1 M KCl and having resistances of about 140 MΩ. The intracellular and reference electrodes were connected to the preamplifier (Mentor N-950, Minneapolis, MN, USA) by $AgCl_2$-coated wires. This system enabled current to be passed through the recording electrode while the voltage drop across the recording system (electrodes and solutions) was balanced by the equivalent of a Wheatstone bridge circuit. This method for recording changes in membrane potential in response to current steps has been much discussed (see, for example, Engel, Barcilon & Eisenberg, 1972). The values of the input resistance and time constant were determined from the amplitude and time course of small (< 10 mV) hyperpolarizing electrotonic potentials. Data were stored on video cassettes in digital form (VR-100, Instrutech Corp.) for subsequent analysis with the aid of a computer software package (pCLAMP, Axon Instruments, Foster City, CA, USA). When the microelectrode was inside a cell, electrotonic potentials were well described by a function consisting of the sum of two exponentials. In three experiments, when the microelectrode was removed to just outside the cell in the extracellular space, the signal was described by a single exponential function, with a time constant of ~1 ms. The faster component of the response recorded inside the cell had a time constant similar to that of the microelectrode. This therefore limited the detection of any fast electrotonic components which may have resulted from any coupling between cells. The means ± S.E.M. of the data for n cells are given throughout.

The following drugs were applied to the preparation via the perfusion system. Hexamethonium chloride (Light & Co., Colnbrook, UK); tetrodotoxin (Calbiochem, San Diego, CA, USA); tetraethylammonium chloride and bethanechol chloride (carbamyl-β-methylcholine chloride; Sigma Chemical Co., St Louis, MO, USA).

RESULTS

Passive electrical properties

Guinea-pig adrenal chromaffin cells had a resting membrane potential of −60·9 ± 0·5 mV (n = 81, from 41 animals). When an impalement was secure there was no evidence of the spontaneous discharge of action potentials. Small (generally less than 6 mV) spontaneous excitatory synaptic potentials (ESPs) were recorded in some cells (see below).

Voltage responses to the injection of brief (40–50 ms) current steps injected through the recording electrode showed that these cells were electrically excitable (Fig. 1*A*). Electrotonic potentials in response to small current steps were averaged and I–V curves gave a value of 180 ± 14 MΩ (n = 23) for the input resistance (R_{in}) at membrane potentials up to 10 mV more negative than the resting potential. Values of R_{in} ranged from 77 to 327 MΩ. The time course of small electrotonic potentials was fitted by a single exponential function. Given the limitations of the 'bridge' technique for passing current through high-resistance microelectrodes (see Methods) we estimated a value of 9·3 ± 0·3 ms (n = 45) for the input time constant (τ_{in}).

During these experiments we also impaled cells with much higher resting potentials of −70 mV or more. These cells had very short input time constants (τ_{in} < 5 ms) and were not electrically excitable. It seems most likely that these were cortical cells (Matthews, 1967).

Action potentials

Every chromaffin cell responded to a depolarizing current step with an action potential. Action potentials initiated by the termination of a hyperpolarizing current step (anode-break excitation) were only observed during the stabilization of an impalement or from cells we considered to be injured (unstable resting membrane potentials and τ_{in} less than 5 ms). Typically, action potentials were initiated by depolarizing current steps of 30–60 pA. In all cells the action potential arose in an 'all-or-nothing' manner and transitional active responses were not observed. The threshold depolarization was found to be 9·5 ± 0·3 mV (n = 63) positive of the resting membrane potential. The duration of the action potential measured midway between the resting potential and the peak of the action potential (half-duration) was 1·56 ± 0·04 ms (n = 63). Repolarization was associated with a fast 'undershoot' during which the membrane hyperpolarized up to 20 mV more negative than the resting potential.

The amplitude of action potentials evoked by direct stimulation had a mean of 61·4 ± 1·4 mV (n = 63). Values varied from 43 to 93 mV, but we had no reason to rule out any data from this sample of impalements since these action potentials arose from cells with similar input resistances and resting membrane potentials. When we applied the criterion that the half-duration of the action potential should be less than 2 ms we found a mean value for action potential amplitude of 62·0 ± 1·8 mV (n = 55), a value which was not significantly different from that determined for the larger population.

When the duration of the current step was increased 10-fold to 500 ms, most chromaffin cells continued to respond with a single action potential that occurred at the onset of the step (Fig. 2*A*). When the strength of the current step was increased, all cells responded with one or more further action potentials (n = 33, Fig. 2*A* and *B*). The

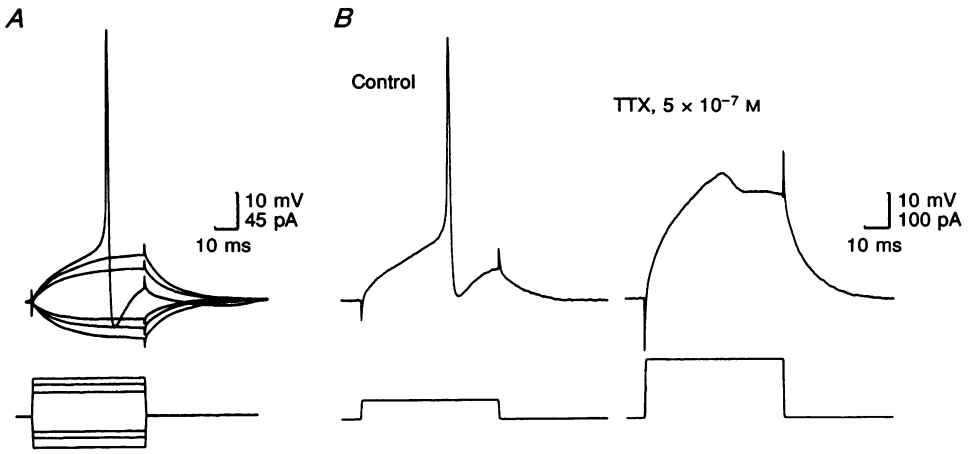

Figure 1. Chromaffin cells have fast, TTX-sensitive action potentials
A, changes in membrane potential in response to a series of hyperpolarizing and depolarizing current steps, 50 ms in duration, from a cell with an R_{in} of 250 MΩ. *B*, action potential recorded from a different cell illustrating the effect of tetrodotoxin (TTX, 5×10^{-7} M). A 4-fold increase in current caused a slower active response.

majority of cells responded to current steps of increasing amplitude as shown in Fig. 2*A* ($n = 25$) with only a brief burst of two or three action potentials at the onset of the step. Thus they behaved in a way that was similar to one class of sympathetic ganglion cells referred to as 'phasic' by Cassell, Clark & McLachlan (1986). Some cells responded to long current steps like that shown in Fig. 2*B*, where an increase in current caused repetitive firing of ten action potentials whose amplitude was maintained. However, this discharge was not maintained during the entire

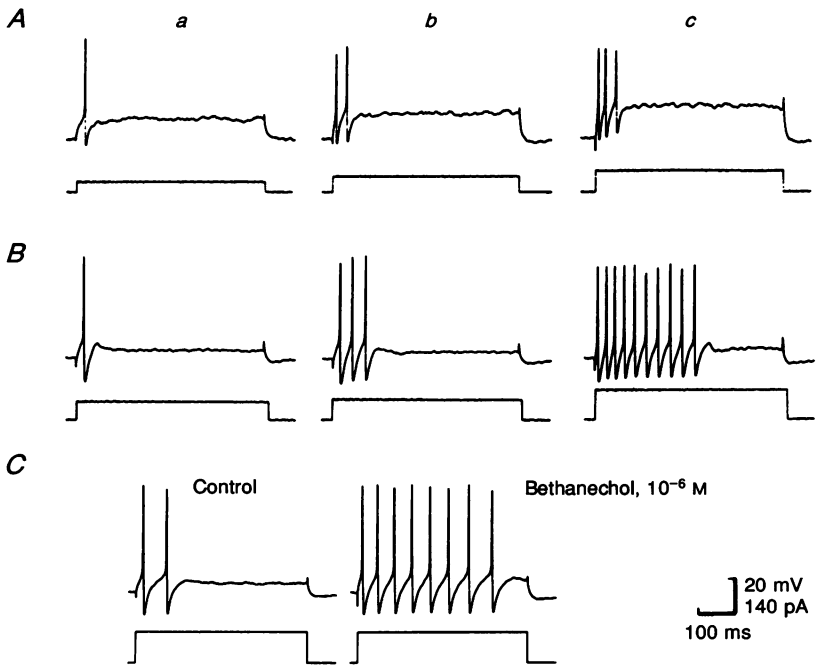

Figure 2. Responses to prolonged current steps of threshold intensity resemble those of phasic ganglion cells
Changes in membrane potential in response to 500 ms current steps. *A* and *B*, responses from 2 different cells to currents of increasing magnitude (*a*, *b* and *c*). Note that increasing the current in *Ac* caused only a burst of 3 action potentials at the onset of the current step, whereas a burst of 10 action potentials occurred following the onset of the step in *Bc*. *C*, an example of the action of bethanecol on responses from a further cell (see text).

duration of the current step. The minimum interval between the first and second action potentials during an initial burst was about 25 ms, similar to that observed in phasic ganglion cells (Cassell *et al.* 1986). It has been shown that the response pattern characteristic of phasic sympathetic ganglion cells could be converted into a tonic response by muscarinic agonists such as bethanechol (Cassell *et al.* 1986). We tested the action of bethanechol, (5×10^{-7} to 5×10^{-6} M) on the responses recorded from fourteen cells (eleven preparations) to a 500 ms current step that was just suprathreshold. In some cells bethanechol caused the discharge of action potentials to continue throughout the current step. In others, such as that of Fig. 2*C*, the discharge of action potentials ceased just before the end of the current step. A small increase in current in this cell caused the discharge to persist throughout the current step (not shown). In two cells, no effect of bethanechol could be detected.

Action potentials were rapidly and reversibly blocked by tetrodotoxin (TTX, 5×10^{-7} M). If the strength of the current step was increased, an active response or 'hump' appeared on the crest of depolarizing electrotonic potentials (Fig. 1*B*). A similar response was observed by Brandt, Hagiwara, Kidokoro & Miyazaki (1976), who recorded from isolated rat chromaffin cells. Prolonged exposure (20 min or more) to high concentrations of tetraethylammonium (TEA) ions (up to 20 mM) increased the amplitude of the TTX-resistant active response, but this was not explored further during the present study.

Responses to nerve stimulation

All cells responded to an adequate transmural stimulus (pulse width up to 0·3 ms, frequency < 0·3 Hz) with an action potential associated with evidence of a variable degree of synaptic activity. In these experiments, the stimulus strength was routinely reduced to below threshold for any detectable synaptic response and then gradually increased. In 42% of cells studied ($n = 76$) the threshold response appeared to be due to the excitation of a pre-synaptic fibre that exerted strong synaptic action (Blackman, Crowcroft, Devine, Holman & Yonemura, 1969). The response was 'all-or-nothing' and it was impossible to grade it by adjusting either the strength or duration of the stimulating pulse. An example of such a response is shown in Fig. 3*A*; after a latency of 7·5 ms from the onset of the stimulus artifact, an action potential arose with little or no evidence of its synaptic origin. The repolarization phase failed to reach the resting membrane potential, presumably due to the continuing action of the transmitter. The action potential was followed by a further hump of depolarization which eventually decayed back to the resting membrane potential. In the example of Fig. 3*A* the total duration of the depolarization from the onset of the response until the return to the resting potential was

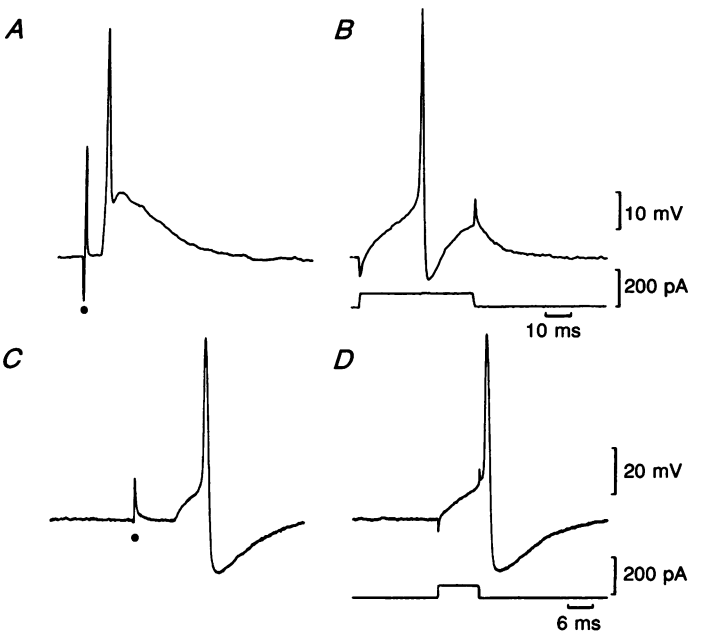

Figure 3. Action potentials evoked by nerve stimulation compared with those evoked by injection of depolarizing current steps
A and *C*, responses to nerve stimulation recorded from two different cells. *A*, an example of a threshold response exerted by a fibre having strong synaptic action; *C*, an example of much weaker synaptic action. *B* and *D*, action potentials in response to depolarizing current steps recorded from the same two cells.

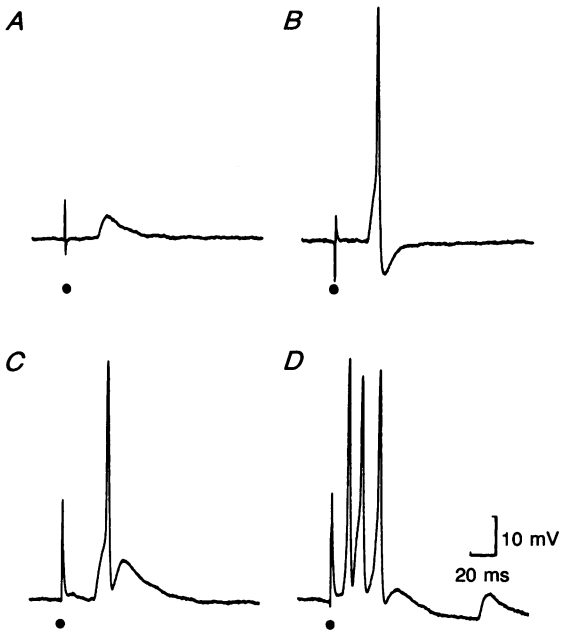

Figure 4. Effects of increasing the intensity of nerve stimulation
Responses recorded from the same cell as the strength of the stimulus was increased progressively. Note that in *D*, a presynaptic fibre was recruited with a relatively short latency.

46 ms. Figure 3*B* shows an action potential evoked in the same cell by a depolarizing current step. In contrast with Fig. 3*A* this action potential was followed by the marked undershoot described previously.

In all but three of the remaining cells the response to a threshold stimulus to the nerve supply was characterized by much less intense synaptic action. In Fig. 3*C* this consisted of an excitatory synaptic potential (ESP) that just exceeded threshold for the initiation of an action potential. Any observable continuing synaptic action was clearly shunted by the conductances associated with the action potential, which may be compared with that in response to direct stimulation in Fig. 3*D*.

Grading of responses to nerve stimulation

An example of the recruitment of input fibres with increases in stimulus strength is shown in Fig. 4. The amplitude of the ESP in Fig. 4*A* fluctuated around threshold (9 mV more positive than the resting potential). Increasing the stimulus intensity ensured that synaptic input always exceeded threshold (Fig. 4*B*). Nevertheless the conductance responsible for the undershoot of the

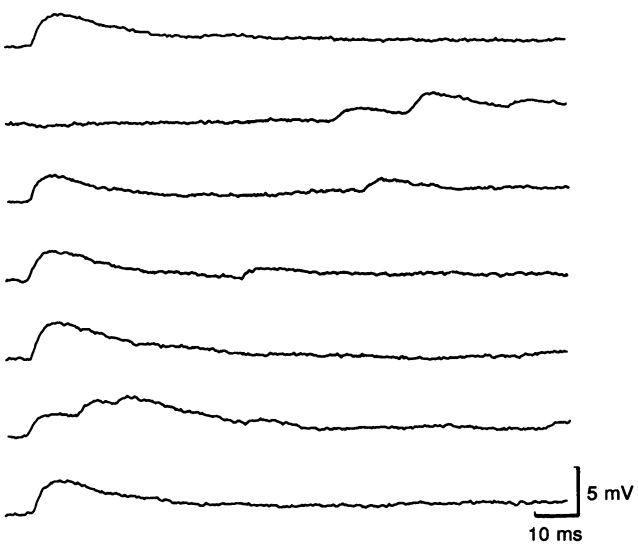

Figure 5. Spontaneous excitatory synaptic potentials
Spontaneous ESPs recorded from a cell showing a relatively high frequency of these events even though the stimulus frequency was 0·3 Hz.

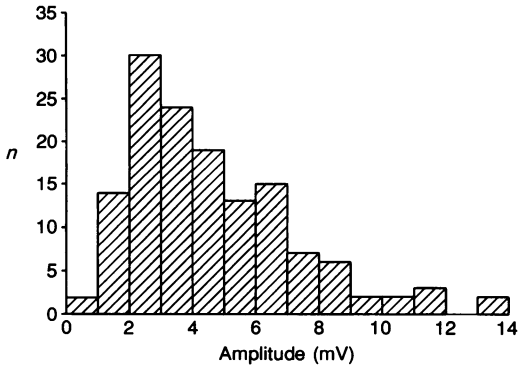

Figure 6. Amplitudes of SESPs
Amplitude histogram of 139 SESPs recorded from 1 cell.

action potential was still effective and no further evidence of synaptic action followed the action potential. Further increases in stimulus intensity produced the responses shown in Fig. 4*C* in which the after-hyperpolarization associated with the action potential did not reach the level of the resting membrane potential. The latency of the response shown in Fig. 4*D* was reduced. The total duration of the depolarization in Fig. 4*D* was around 60 ms and it was sufficiently large to initiate three action potentials. This was the maximal response seen in that cell. Note the occurrence of a spontaneous ESP following the evoked response in Fig. 4*D* and the prolonged after-hyperpolarization that followed the depolarization.

All responses to nerve stimulation were blocked by 1–2 mM hexamethonium ($n = 10$).

Spontaneous ESPs

During the majority of our experiments with low frequencies of stimulation (up to 0·3 Hz) spontaneous ESPs (SESPs) occurred occasionally and were usually superimposed on the decay phase of the evoked ESP. Similar ESPs that were not time-locked to the stimulus were also observed on rare occasions during the interval between successive responses, especially following stimulation of a strong input fibre. The characteristics of these potentials are illustrated in Fig. 5, which shows random sections of records from a cell where a relatively high frequency of SESPs occurred. When higher frequencies of stimulation were used the frequency of such SESPs was greatly increased (see below). In a sample of 117 such SESPs from ten impalements in seven preparations the rise time (10–90% of time to peak) was 3·6 ± 0·1 ms. This value is similar to the rise time (3·8 ms) for synaptic potentials in sympathetic ganglia (Coleman, 1987). Amplitudes ranged from 1 to 14 mV. By analogy with other synapses (notably those on sympathetic ganglia) we have tentatively assumed that the SESPs are caused by the release of quanta of acetylcholine (ACh). Their decay phase was fitted by a single exponential with a time constant of 18·7 ± 0·5 ms ($n = 40$).

Figure 6 is a histogram of the amplitudes of SESPs recorded from one cell and is representative of data obtained so far. The shape of the histogram suggests that the distribution of amplitudes is of the gamma type (Robinson, 1976) rather than a normal distribution.

Components of the response to nerve stimulation with fast rise times

In some cells throughout these experiments we have observed a component of the response to nerve stimulation with a rising phase that was faster than that of ESPs or SESPs (see Fig. 7*B*, *C*, *E* and *F*). The amplitudes of these events ranged from background noise level up to 9·5 mV. Their rise times (10–90% of peak), measured in the same way as that used to analyse SESPs, had a value of 1·75 ± 0·36 ms ($n = 39$). When the rise times of SESPs and of events with fast rise times were recorded in the same

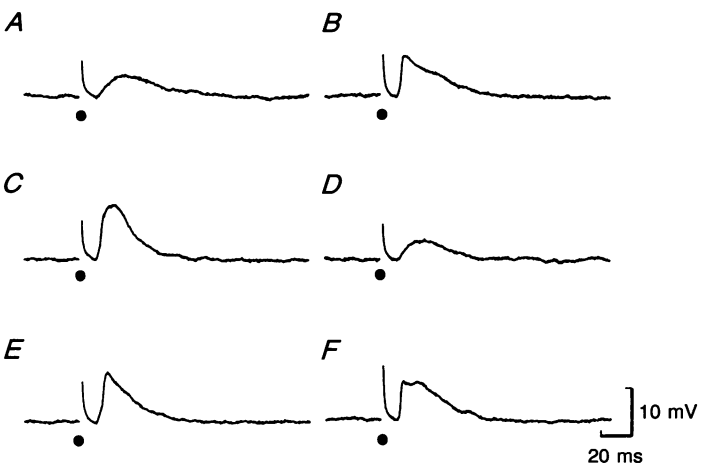

Figure 7. Some examples of subthreshold ESPs and evoked responses with rapid rise times
Responses to transmural stimulation recorded from a cell showing the intermittent occurrence of events with fast rise times (*B*, *C*, *E* and *F*). See text for explanation.

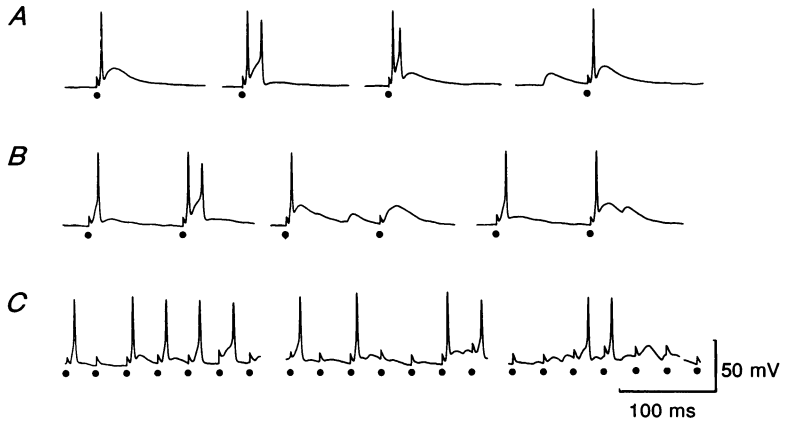

Figure 8. Records showing the response to stimulation at 3, 10 and 30 Hz
Responses recorded from the same cell during repetitive stimulation (600 pulses) at 3 (*A*), 10 (*B*) and 30 Hz (*C*). Records selected towards the conclusion of each stimulus period. See text for explanation.

cell, values of 3·4 ± 0·09 ($n = 32$) and 1·7 ± 0·03 ms ($n = 12$) were found, respectively. These mean values were significantly different ($P < 0·0001$, Student's unpaired t test). In sympathetic ganglion cells events with similar fast rise times (2·0 ms) have been reported and were attributed to distally generated action potentials which propagated passively to the soma (Coleman, 1987).

In general, the level of depolarization reached during the rising phase of these fast events did not immediately decay back to the resting potential. On some occasions there was a brief notch before a maintained period of depolarization (Fig. 7*F*). In this cell the threshold response varied from a fast event followed by depolarization resembling that of the ESP to subthreshold ESPs indistinguishable from SESPs. In three cells events with rapid rise times and associated prolonged depolarization initiated an action potential in response to threshold stimulation. It seems likely that similar responses in most cells were shunted out by ESPs and action potentials.

Effects of repetitive nerve stimulation

During prolonged periods of repetitive stimulation (> 10 min) at frequencies of 0·3, 1·0 and 3·0 Hz, responses followed each stimulus with no evidence of fatigue or desensitization. Individual responses fluctuated (Fig. 8*A*). At 3·0 Hz some cells showed an increase in the frequency

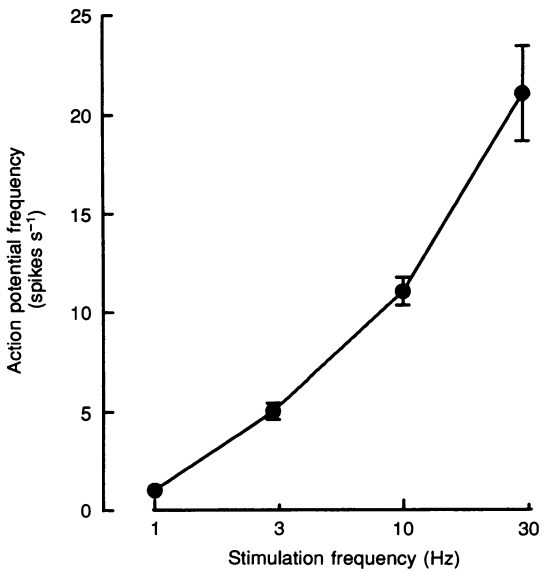

Figure 9. The number of action potentials per second in response to increasing stimulus frequency
Relation between the frequency of stimulation and the initiation of action potentials during repetitive stimulation (600 pulses).

of SESPs. At 10 Hz the frequency of SESPs was clearly increased throughout the period of stimulation and immediately afterwards. At 30 Hz successive SESPs summed with each other to cause a varying level of depolarization that led to a decrease in the amplitude and an increase in the duration of action potentials. At 30 Hz some stimuli failed to evoke a response and this also occurred at 10 Hz in some preparations. We found evidence of an intermittent failure of stimuli to evoke action potentials interspersed with trains of stimuli that caused suprathreshold ESPs. A similar phenomenon was noticed in the guinea-pig superior cervical ganglion during continuous stimulation at 20 Hz (Sacchi & Perri, 1971) and at 10 Hz (Bennett & McLachlan, 1972).

In a series of experiments ($n = 8$ preparations) we recorded responses to repetitive stimulation following the protocol described by Wakade (1981), who measured the release of catecholamines from rat and guinea-pig adrenals in response to 600 stimuli delivered at 0·3, 1·0, 3·0, 10 and 30 Hz. Ten minute periods of rest were allowed between each period of stimulation. Frequencies were selected at random and the polarity of the stimulating electrodes was reversed approximately every 15 s. At 0·3, 1·0 and 3·0 Hz successive responses followed each stimulus with no evidence of fatigue or desensitization. As can be seen from Fig. 8*B* and *C*, action potentials occurred intermittently after each stimulus. They often arose from a depolarized level of membrane potential and appeared to be somewhat inactivated. Upon cessation of stimulation at 10 and 30 Hz, cells continued to show an increased frequency of SESPs. The relation between action potential frequency and stimulus frequency for 600 stimuli is shown in Fig. 9.

DISCUSSION

Properties of chromaffin cells *in situ*

There has been some debate about the value of the resting membrane potential of adrenal chromaffin cells (Nassar-Gentina, Pollard & Rojas, 1988). We feel that the value of −60 mV for guinea-pig cells *in situ* is reliable since action potentials of 70 mV or more were recorded during impalements that lasted for up to 60 min in some cells. We estimate that the value of the input resistance was about 200 MΩ. We are aware of the problems involved in determining this value when a single high-resistance microelectrode is used to pass current into the cell and changes in membrane potential are assessed by a bridge method (see Methods). Nevertheless we found no evidence during any impalement for an input resistance in the gigaohm range that is generally observed for freshly isolated or cultured chromaffin cells with patch electrodes. The value of input capacitance (C_{in}) calculated from R_{in} and τ_{in} was about 50 pF. This is nearly an order of magnitude greater than that found for small (12–16 μm), isolated chromaffin cells by Fenwick *et al.* (1982*a*). If a value for capacitance of 1 μF cm^{-2} is assumed and the diameter of the cells we impaled successfully (presumably large) was about 25 μm then C_{in}, calculated from surface area, should have been around 20 pF. The value of 50 pF suggests that we were recording from an area of membrane in excess of that corresponding to a single cell. Although some leakage around the microelectrode cannot be ruled out, weak electrical coupling between neighbouring cells could contribute to the low value of R_{in} (see Nassar-Gentina *et al.* 1988) as well as the large value of C_{in}. The freeze-fracture study of intercellular junctions in the adrenal medulla by Grynszpan-Winograd & Nicolas (1980) would not appear to discount this possibility.

Such coupling might also explain our observation of small depolarizing events with rapid rise times. We tentatively suggest that the rising phase of these events is caused by the initiation of an action potential in one or more neighbouring cells. When they could be detected, events with rapid rise times were nearly always followed by a phase of relatively long-lasting depolarization resembling that of an ESP. The simplest explanation for the continuing depolarization would seem to be that this was due to strong synaptic input onto a neighbouring cell (or cells). Clearly we need to find direct evidence for or against the existence of weak electrical coupling between chromaffin cells *in situ*.

The threshold value of membrane potential for the initiation of an action potential in response to direct stimulation was about 10 mV more positive than the resting membrane potential. This is half the depolarization required to initiate an action potential in sympathetic ganglion cells with similar membrane potentials (Cassell *et al.* 1986). In the presence of TTX none of the cells studied so far gave an 'all-or-nothing' Ca^{2+} action potential like those observed in myenteric after-hyperpolarizing neurones (Hirst, Holman & Spence, 1974) and in some pelvic ganglia (Davies, Coleman & Parkington, 1993). In reviewing the results of studies done prior to 1981, Kidokoro, Miyazaki & Ozawa (1982) concluded that 'voltage-dependent Ca influx is augmented by depolarization caused by Na inward current during action potentials.' This may also be the case for guinea-pig cells *in situ*.

Responses to prolonged current steps (500 ms) were very similar to those of sympathetic ganglion cells which were classified by Cassell *et al.* (1986) as phasic neurones. These responses are typical of the ganglion cells of the caudal lumbar sympathetic chain that cause vasoconstriction in the hindlimb. These neurones give one or a brief high frequency burst of action potentials at the onset of the current step. Cassell *et al.* (1986) found that the addition of the muscarinic agonist bethanechol converted this phasic behaviour into a tonic discharge and suggested that an M-current terminated the firing in control solution. We found that bethanechol, at concentrations up to 5 μM, was effective in producing tonic firing in some guinea-pig chromaffin cells but to a variable degree. Bethanechol did not appear to cause depolarization of the cells in our

preparations. It is clear that more work is needed to clarify the role of muscarinic receptors in guinea-pig chromaffin cells *in situ*.

Responses to nerve stimulation

Our results confirm those of a recent study by Iijima *et al.* (1992) who used a large photodiode array in combination with a voltage-sensitive dye to study synaptic activation of slices (200–300 μm thick) of rat adrenal gland. Thus we found that synaptic transmission to guinea-pig chromaffin cells in response to a single pulse was always sufficiently intense to evoke an action potential. In 42% of cells it was possible to demonstrate that excitation of a single preganglionic fibre released sufficient ACh to cause intense and prolonged depolarization lasting for a total of up to 70 ms, comparable with that found by Iijima *et al.* (1992). Such strong synaptic action caused the firing of one and occasionally two action potentials in our experiments. During impalements where the 'threshold' response to nerve stimulation indicated the excitation of a preganglionic fibre with weaker synaptic action, an increase in stimulus strength always caused the recruitment of at least one other preganglionic fibre, again leading to strong synaptic input onto that cell and the initiation of one or more action potentials.

In most cases we could distinguish at least two or three different components of a maximal response that could be attributed to at least two or three different fibres with clearly distinguishable thresholds. In an electron-microscopic study of rat adrenal medulla, Coupland (1965) found that chromaffin cells received relatively few synapses. He estimated that there were about three per chromaffin cell. Iijima *et al.* (1992), also working on rats, found evidence that chromaffin cells were organized into clusters of about 100 cells that functioned as units and that each cluster was innervated by about four fibres. The concept of clusters of cells or 'complexes' as the functional units of the adrenal medulla with common innervation was developed initially by Hillarp (1946) and this idea would fit well with our proposal for weak electrical coupling between clumps of neighbouring cells.

During a maximal response in our experiments it was clear that synaptic action and the opening of nicotinic channels was prolonged. Thus in Fig. 4 depolarization did not begin to decay until 46 ms after the onset of the response and an SESP occurred 55 ms later. We suggest that the activation of synapses in the adrenal medulla causes a relatively prolonged increase in the probability of release of quanta of ACh. The occurrence of SESPs as a consequence of nerve excitation was especially marked during and after repetitive stimulation, a phenomenon also noted for guinea-pig pelvic ganglia by Blackman *et al.* (1969).

In every cell, the decay phase of SESPs was slower than that of electrotonic potentials of similar magnitude. This observation is in accordance with evidence that neuronal nicotinic channels have long open times (Fenwick *et al.* 1982*a*; Mathie, Cull-Candy & Colquhoun, 1987; Inoue & Kuriyama, 1991).

We failed to find any evidence for the desensitization of nicotinic receptors by nerve-released ACh. Prolonged stimulation at 10 Hz (up to 40 min in three cells) continued to cause ESPs that were both non-stimulus-locked and stimulus-locked. Action potentials continued to occur, although there were intermittent failures in the responses to nerve stimulation. We have no explanation as to why Wakade (1981) found no increase in the release of catecholamines from adrenal glands from rats and guinea-pigs in response to 600 stimuli at 10 and 30 Hz compared with release at 3 Hz. Using a similar protocol, we found a monotonic increase in action potential frequency (Fig. 9). Kayaalp & McIsaac (1969) measured catecholamine release from the left adrenal gland of dogs in response to ipsilateral splanchnic nerve stimulation. They found a linear relation between the frequency of stimulation (3, 10 and 20 Hz for 1 min) and catecholamine release, as we found for the relation between stimulus frequency and action potential frequency in guinea-pigs.

In summary, we have found many similarities between the responses of guinea-pig chromaffin cells *in situ* to splanchnic nerve stimulation and those recorded from sympathetic ganglion cells from the same species. We suggest that cells may be weakly coupled together into functional complexes and that this results in a reduction of their input resistance, which may be the reason why we failed to observe any action potentials in the absence of splanchnic nerve stimulation. We found no evidence for the desensitization of nicotinic receptors in response to ACh released in response to nerve stimulation.

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