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BURST-PATTERNED STIMULATION PROMOTES NICOTINIC TRANSMISSION IN ISOLATED PERFUSED RAT SYMPATHETIC GANGLIA

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SUMMARY

1. Intracellular recordings of small nicotinic excitatory postsynaptic potentials (EPSPs) were made from rostral cells in superior cervical ganglia (SCG) of rats during and after test stimulation of small preganglionic fibre bundles, while perfusing the isolated ganglia via their arterial vasculature. Perfusion, in contrast to superfusion of desheathed ganglia, (*a*) produced much more rapid and complete equilibration of drugs and ions at synaptic sites, (*b*) greatly reduced depression of EPSPs during high-frequency stimulation, and (*c*) largely prevented slowing of conduction, presumably by minimizing accumulation of K^+ in the intercellular spaces surrounding these sites.

2. Preganglionic inputs were found to fall into two major groups: those in which the EPSP amplitude during 200 pulse trains was facilitated and others in which it was depressed as stimulation frequency in the train was increased from 2 to 20 Hz or from 0·2 to 1·25 Hz. Both the facilitation and the depression were presynaptic, since they occurred without changes in miniature EPSP amplitude.

3. The maximum maintained facilitation was reached at 5–10 Hz with a value 1·26 times the 1·0 Hz control. This was associated with an increase in the binomial parameter n. While long 20 Hz trains produced a similar facilitation to an early plateau, and an increase in n, EPSP amplitude declined as the train progressed. This was associated with a decrease in the binomial parameter p.

4. Unlike the 20 Hz trains, stimulation with 0·5 s long, 20 Hz bursts given every 8 s produced a marked potentiation in facilitating units and this was maintained for as long as the stimulation was continued (3–11 min). Burst-patterned potentiation was 1·66 times larger than the facilitation evoked by tonic stimulation at the same average frequency (1·25 Hz), and more than twice that achieved with long, 200 pulse trains. The potentiation was associated with increases in both n and p in the first EPSP of the burst and mainly with an increase in n at the end of the burst. Potentiation persisted unchanged for about 30 s following the return to control 0·2 Hz stimulation, before declining to control levels over the next 2–3 min. Depressing units on average showed neither burst-patterned potentiation nor post-burst-patterned potentiation.

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5. All inputs tested in Locke solution in which Ca^{2+} was reduced to 0·5 mM with addition of 1·2 mM-Mn^{2+} or 3·8 mM-$MgCl_2$ exhibited a pronounced facilitation within each burst but no extension of potentiation into ensuing bursts. Both burst-patterned potentiation and the post-burst-patterned potentiation were abolished.

6. It is concluded that the burst-patterned form of input to facilitating units in sympathetic ganglia is the most effective means of producing a maintained increase in the quantal content of EPSPs. It is proposed that a persisting potentiation following each burst sums to produce the maintained burst-patterned potentiation which involves the maintenance of the binomial parameters n and p at high levels.

INTRODUCTION

Trains of high-frequency activity have been found to enhance transmission at low quantal content in the vertebrate neuromuscular junction (Del Castillo & Katz, 1954; Liley, 1956), and at low and normal quantal content in neuronal synapses of both autonomic ganglia (Martin & Pilar, 1964; McLachlan, 1975*a*) and of central neurones (Kuno, 1964). High-frequency stimulation also produces an after-potentiation at both low and normal quantal content, lasting from 1 to 2 s of 'facilitation', to minutes of 'potentiation', to hours of 'long-term potentiation' (Larabee & Bronk, 1947; Del Castillo & Katz, 1954; Martin & Pilar, 1964; Brimble, Wallis & Woodward, 1972; Dunant & Dolivo, 1968; Bliss & Lomo, 1973). At normal quantal content, however, although short high-frequency trains facilitate transmission, long trains often depress it (Thies, 1965; McLachlan, 1975*b*). In this report the term 'facilitation' is used to refer to enhancement of transmitter release that occurs during a stimulus train, and 'potentiation' to the enhancement that follows a train.

Studies of activity in preganglionic units innervating mammalian cervical sympathetic ganglia have revealed the existence of bursts of activity in phase with inspiration in approximately half of the units (Preiss, Kirchner & Polosa, 1975). Under combined conditions of hypercapnia and hypoxia these units fire with 10–40 Hz bursts during inspiration separated by periods of relative silence during expiration (Bachoo & Polosa, 1987). Birks (1978, 1979, 1982) has shown in the perfused cat superior cervical ganglion (SCG) that repetitive stimulation with short high-frequency bursts at intervals of several seconds produces a larger potentiation of acetylcholine (ACh) release than continuous stimulation at the same frequency, and that this potentiation is stable during many tens of minutes of stimulation. Other studies have shown for instance that the release of vasopressin and oxytocin from the neurohypophysis is also potentiated by the burst-patterning form of stimulation (Dutton & Dyball, 1979; Bichnell, Flint, Leng & Sheldrick, 1982).

While several studies have shown that autonomic effector activation can be potentiated by burst patterning of presynaptic activity (Edwards, 1982; Grundy & Scratcherd, 1982; Andersson *et al.* 1982; Bloom *et al.* 1984) these studies did not identify whether the enhancement was at the level of transmitter release from the stimulated axons or of the postsynaptic integration of the excitatory activation. A study on the cat stellate ganglion *in vivo*, using the post-ganglionic nerve compound action potential as an indirect index of the level of transmission (Birks, Laskey &

Polosa, 1981), suggested that burst-patterned stimulation of the preganglionic nerve produces levels of post-ganglionic neuronal recruitment corresponding to the potentiated levels of ACh release observed by Birks (1982) in the perfused cat SCG. The effects were contaminated by depression of the compound action potential however, and so left in question whether potentiated transmitter release was synaptically active throughout the burst.

In the present study the dependence of nicotinic transmission on the frequency and pattern of presynaptic activity has been examined using intracellular recording in isolated and perfused rat SCG. Burst-patterned activity was found to potentiate all the EPSPs in the bursts by increasing the number of ACh quanta released and this potentiation was maintained throughout the stimulation. Furthermore burst-patterned activation was found to be the most effective means of increasing quantal content of EPSPs during activity. A binomial analysis was carried out in order to evaluate which statistical parameters (n, p) were sensitive to the pattern of stimulation.

METHODS

Preparation. Male Sprague–Dawley rats weighing 200–250 g were housed for at least 1 week before experimentation. Rats were then placed in a dark Plexiglas box to adapt for 30 min. Anaesthesia was induced with 2% halothane in a gas mixture of 35% O_2 and 65% N_2O. Intraperitoneal injection of 0·75 g/kg urethane was then administered to maintain anaesthesia while the tissue was excised for *in vitro* experimentation. The animals were killed with an additional injection of 2·5 g/kg urethane.

Perfusion. In view of earlier findings of considerable accumulation of extracellular K^+ during trains of moderate- to high-frequency activity in desheathed and superfused rat ganglia *in vitro* (Galvan, ten Bruggencate & Senekowitsch, 1979) we perfused ganglia through their arterial vasculature. The effect of a solution exchange occurred more quickly, and was more complete when the solution was introduced via arterial perfusion to ganglia with the sheath intact, rather than in a brisk superfusion to desheathed ganglia. The clearest examples of this were seen with blockers of ganglionic transmission such as hexamethonium and low Ca^{2+}.

Figure 1 shows a typical time course of the wash-in of the effects of 0·2 mM-hexamethonium on the post-ganglionic nerve compound action potential (CAP) recorded from the internal carotid nerve during preganglionic stimulation at 1 Hz in a perfused preparation and 0·2 Hz in a non-perfused preparation. In the perfused ganglion complete block of the CAP occurred over a period of 15 s following the start of a noticeable decline in amplitude. In the non-perfused ganglion, however, the same concentration of hexamethonium produced a block with a much slower onset, which did not become complete even after many minutes. Increasing the superfusion rate did not facilitate the exchange. The time course of recovery from blockade was similar. The time courses of block and recovery were equally rapid when the Locke perfusion was temporarily switched to low Ca^{2+}.

This large difference between these two methods implies that the extracellular fluid around cells and cell processes located throughout the ganglion is much better exchanged with the Locke solution when ganglia are perfused. All the experiments therefore were performed on perfused ganglia, with the exception of one set performed on ganglia that were desheathed and superfused to compare transmission during burst-patterned stimulation in perfused and non-perfused ganglia.

In both the perfused and non-perfused situations the ganglia were placed in a 0·4 ml bath, superfused at 2 ml/min with Locke solution to exchange the bath volume 5 times/min. In all experiments the bath and the Locke solution were held at $37·0 \pm 0·2$ °C. In the case of non-perfused ganglia the ganglionic sheath was completely removed. In the case of perfused ganglia the sheath was left intact except for a small slit near the rostral pole, where no superficial blood vessels were located, to allow insertion of the microelectrode. Perfusion was with the same Locke solution via the common carotid artery at a pressure of 110–120 mmHg, which yielded flow rates of 0·1–0·3 ml/

min. To achieve successful perfusion, the common carotid artery was cannulated and the internal carotid artery and all but one of the major branches of the external carotid artery were tied off at points peripheral to the ganglion. The final major branch of the external carotid artery was tied off only after all residual blood in the carotid arteries was washed out by the perfusion solution. The ganglion was then excised together with stretches of pre- and post-ganglionic nerves.

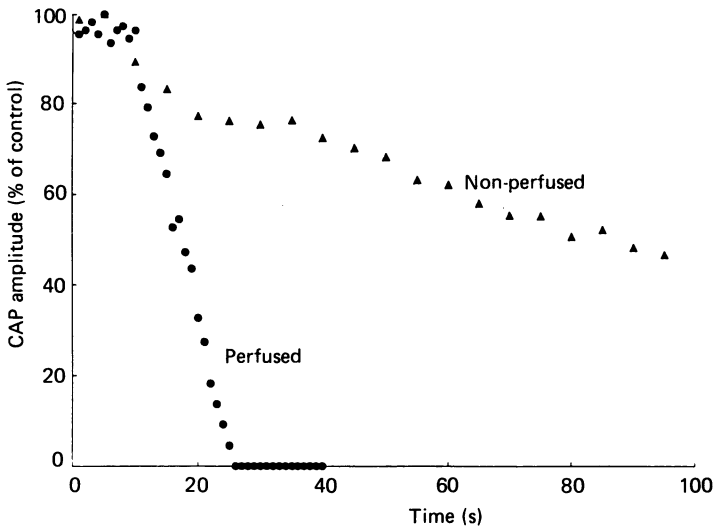

Fig. 1. Effect of introducing a ganglionic blocking agent by vascular perfusion and by superfusion. Small bundles of preganglionic axons were stimulated at intensities that were twice maximal. The compound action potential (CAP) was recorded from the postganglionic internal carotid nerve. At time zero the bathing solution was switched from Locke solution to Locke solution containing 0·2 mM-hexamethonium. In a desheathed ganglion that was superfused in the conventional manner (▲), the onset of blockade was slow, reaching about 50% in 1·5 min. In a ganglion that was perfused via the common carotid artery, and which had its sheath intact except for a small hole made near the rostral pole to admit microelectrodes (●), the onset of the block was very rapid, with complete block being attained within 15 s.

Solutions. Standard Locke solutions consisted of (in mM): NaCl, 129; KCl, 5·6; $NaHCO_3$, 25; $CaCl_2$, 2·2; $MgCl_2$, 1·2; choline chloride, 0·05; and dextrose, 8. The solutions were bubbled with 95% O_2–5% CO_2 (pH 7·4) and delivered under pressure from a tank containing the same gas mixture. Hexamethonium bromide was obtained from Sigma. Changes in the Ca^{2+} concentration of the bathing solutions were made in both the perfusion and the superfusion solutions.

Stimulation and input selection. Small bundles of preganglionic axons were teased from the preganglionic nerve and stimulated 2–5 times supramaximally with suction electrodes placed approximately 2 cm from the entry point of the nerve into the ganglion. At the beginning of each experiment, once the recording bath had equilibrated to 37 °C, the preganglionic bundles were tetanized with 200–800 pulses to 20 Hz in order to activate maximally long-term potentiation, which has been shown to be sustained for more than 14 h with no appreciable decay at physiological temperatures in rat SCG *in vitro* (cf. Briggs, Brown & McAfee, 1985). This procedure was performed in an attempt to ensure that the tetanic and post-tetanic events studied would not be contaminated by long-term potentiation. None the less in six of the sixty inputs examined in normal Ca^{2+} there was a long-lasting potentiation of EPSP amplitude following high-frequency activity, such that EPSP amplitude did not return to control values within the typical 1–4 min recovery. These inputs were excluded from the present analysis.

Under the stimulus conditions used here, which activated only small numbers of preganglionic

fibres, the slow synaptic events studied in detail in rat SCG by Brown & Selyanko (1985) were present in only five of all the cells studied, and then only at stimulation frequencies of 10 Hz and greater. In the rest of the cells, membrane potential and input resistance remained constant throughout the range of stimulation frequencies. The five cells in which this was not the case were rejected in order to ensure that the driving force remained constant throughout the test protocol. Excluding these the total number of inputs studied in normal Ca^{2+} was forty-nine.

Compound action potentials (CAPs) recorded from the post-ganglionic nerve were used to evaluate the stability of the preparation and CAPs recorded from the preganglionic nerve near its entry point into the ganglion were monitored to ensure that the preganglionic nerve followed faithfully the stimulation frequency. Inputs which evoked subthreshold EPSPs ranging from 1·4 to 15 mV, in cells with resting potentials of −50 to −75 mV and input resistances of 25–100 MΩ, and action potential (AP) overshoots of 15–20 mV were selected for study. Some cells were hyperpolarized by constant-current injection of 0·1–1·0 nA to membrane potentials ranging from −85 to −95 mV in order to increase the signal-to-noise ratio for measurements of miniature EPSPs (MEPSPs) and to prevent potentiated EPSPs from discharging the cell.

Recording and analysis. Microelectrodes (50–90 MΩ) of Frederick Haer borosilicate glass were pulled on a Brown and Flaming puller and filled with 3 M-KCl. Bridge recordings were amplified by an Axoclamp 2A, low-pass filtered at 3 kHz, digitized at 11 kHz along with trigger pulses using a Neuro-Corder DR-484 and stored on VHS video tape using a Mitsubishi HS-328UR recorder. AC recordings of CAPs were amplified with Grass P15 preamplifiers. The records were reconverted to analog signals for analysis and triggered samples were low-pass filtered at 1 kHz and digitized at 4 kHz using a Data-Translation board and an IBM personal computer. EPSP amplitudes were measured by computer. No corrections were made for non-linear summation (cf. McLachlan, 1978). Recordings were played back onto a Nicolet 3091 digital oscilloscope for measurements of the amplitudes of MEPSPs and of latency to EPSP take-off and for verification of computer measurements of EPSP amplitude. In some cells giant MEPSPs were observed, which were 2- to 3-fold larger than the majority of the MEPSPs and in some cases larger than the evoked EPSPs. These were excluded from the MEPSP distributions (cf. Blackman, Ginsborg & Ray, 1963). The MEPSPs were sampled only when they occurred in the interval between evoked EPSPs so that they took off from resting potential.

Estimates of the binomial parameters were made using the equations

$$p = 1 - S^2/mx^2 + s^2/x^2$$

and

$$n = m/p,$$

where S^2 is the variance of EPSP amplitude; x is MEPSP amplitude; m is EPSP amplitude/x; and s^2 is the variance of MEPSP amplitude. The standard errors of the estimates were calculated by computer using eqns (19) and (20) in McLachlan (1978). Estimations were rejected if the standard error exceeded 20%. Theoretical normal and γ-fits to the observed amplitude distributions of MEPSPs were made using eqns (21) and (23) in McLachlan (1978), and, depending on which provided the better fit using the χ^2 test (cf. McLachlan, 1975*a*), one of these was used to construct binomial fits to the amplitude distributions of EPSPs using eqns (22) and (24) (McLachlan, 1978).

RESULTS

Effects of stimulus frequency on quantal content of EPSPs

Saachi & Perri (1971) have described the existence of both facilitating and depressing inputs in guinea-pig SCG, though depressing inputs were not mentioned by McLachlan (1975*a*) or by Bennett, Florin & Pettigrew (1976) who were also working in guinea-pig SCG.

We examined first the responses to repetitive stimulation by stimulating small bundles of preganglionic axons with trains of 200 stimuli at 2–20 Hz, preceded and separated by 5 min rest periods of 1 Hz stimulation, to provide a reference point for each of the higher frequencies. As shown in Fig. 2, EPSP amplitude was facilitated

in about half the inputs ($N = 10$) and depressed in most of the remaining inputs ($N = 13$) following the switch to a higher frequency of stimulation. The two types of input evoked EPSPs of similar amplitudes at the same range of membrane potentials. Moreover, in some instances they were found to activate the same cell indicating that the facilitation and depression were due to presynaptic and not to postsynaptic events. Thus the ganglia contain two types of input which will be referred to as facilitating and depressing inputs.

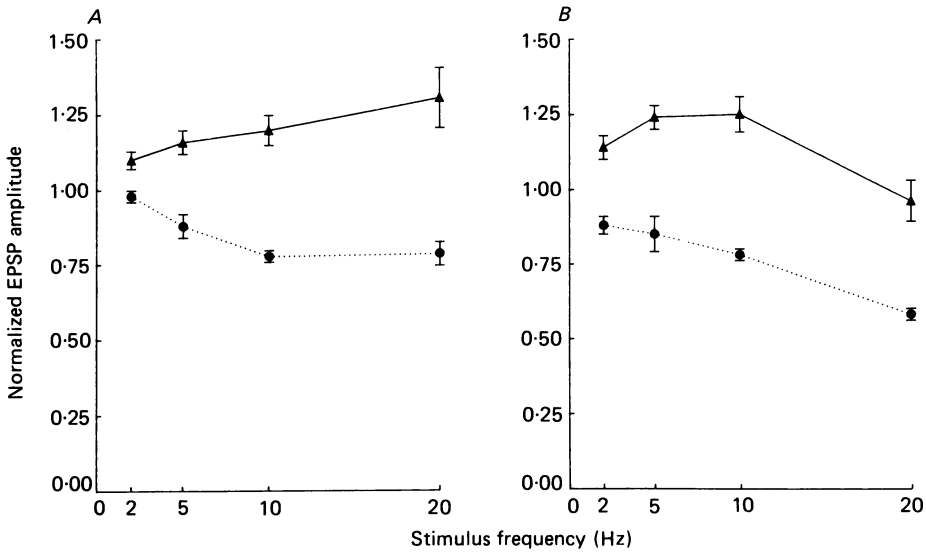

Fig. 2. Frequency dependence of EPSP amplitude in perfused ganglia during trains of 200 stimuli. Stimulation was switched from 1 Hz to 2, 5, 10 or 20 Hz with 5 min rest intervals at 1 Hz, and EPSPs were collected early in the train (pulse numbers 5–20; *A*) and at plateau late in the train (pulse numbers 100–200; *B*) and normalized relative to EPSP amplitude at 1 Hz. Increasing stimulation frequency produced a facilitation in about half the inputs (▲, $N = 10$) and a depression in the remaining ones (●, $N = 13$). Values are expressed as mean ± S.E.M. The differences between depressing and facilitating inputs were significant at all frequencies in both *A* ($P < 0{\cdot}01$) and *B* ($P < 0{\cdot}001$). Note that at frequencies above 10 Hz facilitation was absent late in the train.

Early in a train of stimulation to facilitating inputs EPSP amplitude increased in proportion to the increase in stimulus frequency, reaching a maximum of $1{\cdot}31 \pm 0{\cdot}10$ times control at 20 Hz, the highest frequency tested (Fig. 2*A*). The early facilitation at 20 Hz reached a plateau in five to ten pulses. An example is shown in Fig. 4*A* as the averaged response of the first ten EPSPs in six consecutive 20 Hz trains given at 5 min intervals. There was a tendency towards further increase in EPSP amplitude as the trains progressed at all frequencies except 20 Hz. Here there was a decline in the next 50–100 pulses which reached a plateau of $0{\cdot}96 \pm 0{\cdot}07$ times control in the latter half of the train (Figs 2*B* and 5*C*). The maximum maintained potentiation was reached at 5–10 Hz, at a value 1·26 times the 1 Hz control (Table 1). A facilitation to 1·08 was also evident when stimulation was switched from 0·2 to 1·0 Hz (Table 1).

In the depressing inputs, EPSP amplitudes in thirteen experiments decreased

early in the train, most noticeably at 20 Hz (Fig. 2*A*), and then tended to stabilize late in the train, with a maximum depression to 0·51 ± 0·02 times the 1 Hz control at 20 Hz (Fig. 2*A* and *B*, ●). The differences between the mean amplitude of EPSPs evoked by facilitating and depressing units was significant both early ($P < 0·01$) and late ($P < 0·001$) in the trains at all frequencies. In contrast to the facilitating inputs, a depression to 0·84 was observed on switching from 0·2 to 1·0 Hz. The two types of

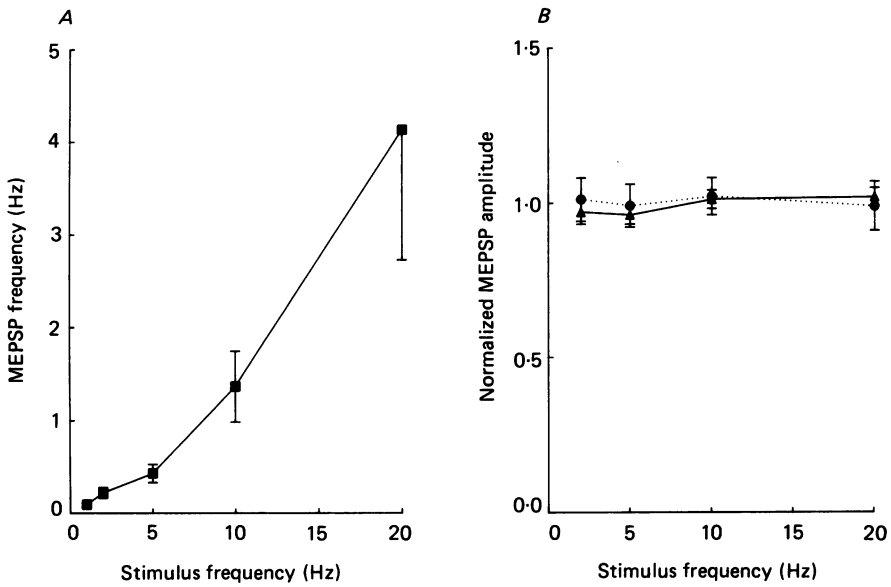

Fig. 3. The dependence of MEPSP frequency and amplitude on the frequency of preganglionic stimulation at plateau. Increasing stimulus frequency evoked increases in MEPSP frequency (*A*), with no significant change in amplitude (*B*). The data from six facilitating inputs (▲) and five depressing inputs (●) shown separately in *B* were pooled in *A*. In *B* MEPSP amplitudes were normalized with respect to the amplitudes of MEPSPs recorded during stimulation at 1 Hz. Values are mean ± S.E.M. These results show that changes in EPSP amplitude in both groups in Fig. 2 were due to changes in the quantal content of release.

input could therefore be identified by the change in average EPSP amplitude on switching from 0·2 to 1·25 Hz.

Measurements of MEPSPs were made at the late plateau at all frequencies using only those MEPSPs which took off from resting membrane potential between the evoked EPSPs. Figure 4*B* shows sample records for one facilitating input during and immediately after a 20 Hz train. The MEPSPs which were used for the amplitude distributions could easily be resolved and the ones which were superimposed on the EPSPs were rejected.

The frequency of the spontaneous MEPSPs increased by as much as eighty times with increases of stimulus frequency from 1 to 20 Hz (Fig. 3*A*) in both facilitating and depressing inputs. Since the MEPSP frequency in unstimulated ganglia was usually less than 0·05 Hz ($N = 50$) the MEPSPs that were collected during stimulation could reasonably be assumed to arise from spontaneous release of quanta

specifically from the stimulated axons. The amplitude of the MEPSPs remained constant throughout the frequency range (Fig. 3*B*). Changes in EPSP amplitude shown in Fig. 2 were therefore due to changes in the quantal content (m) of release.

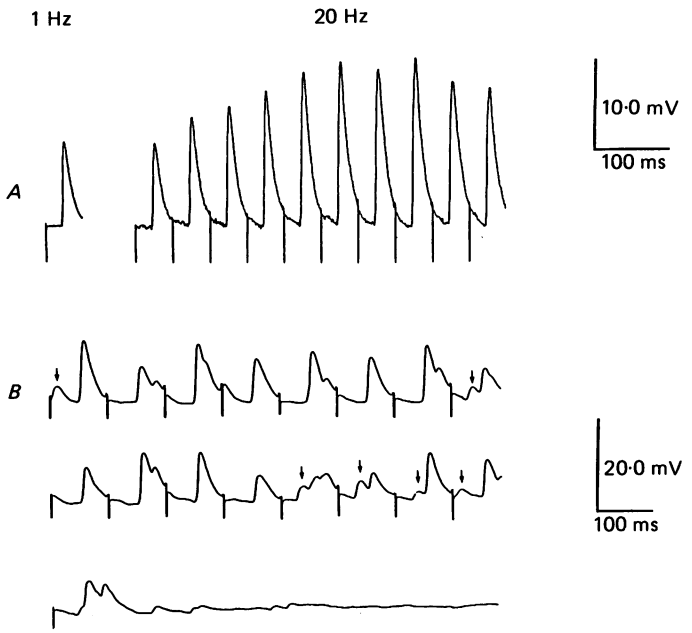

Fig. 4. *A*, increase in amplitude of EPSPs evoked by a facilitating input early in a 20 Hz train. Average record of the first ten pulses of five consecutive 20 Hz trains for an input with a large facilitation. Trains were separated by 5 min rest periods of 1 Hz stimulation to allow for recovery. An averaged record of thirty EPSPs evoked during the 1 Hz stimulation before each of the five trains ($N = 150$) is shown for comparison. EPSP amplitude reached a peak by the sixth pulse in the train and then decreased slightly to the early plateau analysed in Fig. 5. *B*, occurrence of MEPSPs during and after a 20 Hz train to a facilitating input. Consecutive single sweeps at the end of a 20 Hz train in the same experiment as *A*. Only MEPSPs taking off from baseline (arrows) were included in the binomial analysis and these were easily resolved during the train (top and middle records) and after the end of the train (bottom record). In *A*, as in Figs 6 and 9, computer averages of records low-pass filtered at 1 kHz are shown. In *B* single sweeps were filtered at lower frequencies for the *X*–*Y* plotter. Measurements of EPSP and MEPSP amplitude were done at higher gain on the digital oscilliscope after filtering at 1 kHz (see Methods). Resting membrane potential was −87 mV.

Binomial analysis of the EPSP amplitudes early and late in the 5 and 20 Hz trains was carried out on the recordings in which stable steady states were obtained (Fig. 5). In the case of facilitating inputs, the changes in m at 5 Hz and at the early plateau at 20 Hz were associated with increases in n (Table 1). Similar results have been described for the majority of inputs to guinea-pig SCG (McLachlan, 1975*a*; Bennett *et al.* 1976). At the late 20 Hz plateau the decline of m was accompanied by decreases in p. In the depressing inputs, n was decreased at 5 Hz and early in the 20 Hz trains and an additional decrease of p occurred late in the 20 Hz trains.

Effects of burst-patterned stimulation on quantal release

Preganglionic stimulation of the perfused cat SCG with 0·5 s 40 Hz bursts at intervals of 8 s has been shown to produce a marked potentiation of ACh output when compared to tonic stimulation with equally spaced pulses at the same average

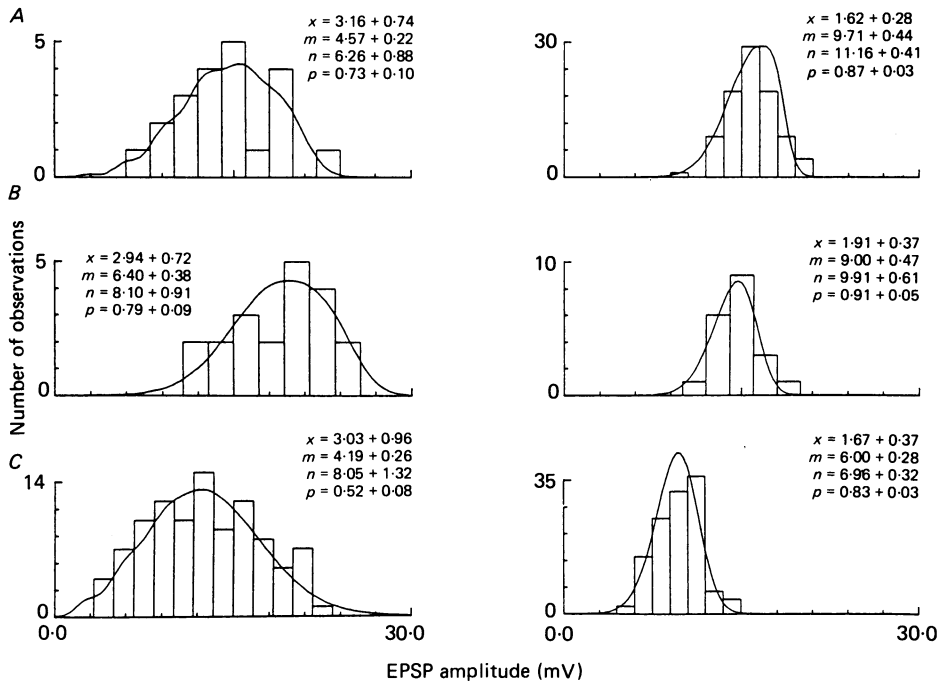

Fig. 5. Binomial analysis of facilitation and depression of quantal release following a switch in stimulation frequency from 1 to 20 Hz in one facilitating input and one depressing input. Histograms of the amplitude distributions are shown for steady-state EPSPs at 1 Hz(*A*), and for EPSPs at 20 Hz, both early (pulses 5–25; *B*) and late (pulses 100–200; *C*) in the train. Values for mean MEPSP amplitude, *m*, *n* and *p* are given for each distribution. In both inputs, *p* decreased during the trains, but *n* increased early and remained high late in the train in the facilitating input, and decreased early, and even more so late in the train in the depressing input. Resting membrane potential was −70 mV for the facilitating and −57 mV for the depressing input. The continuous lines in *A*, *B* and *C* represent theoretical binomial distributions constructed from the estimated values of *n* and *p*.

frequency (Birks, 1979, 1982), and this potentiation was maintained for over 60 min of burst-patterned activation. The effect of burst-patterned stimulation on the amplitude of nicotinic EPSPs was therefore examined to determine its influence on transmission. As before, small preganglionic bundles were stimulated. Stimulation was at 0·2 Hz for 5 min periods before and after 3–5 min of tonic stimulation at 1·25 Hz, followed by 3–5 min (about the same number of pulses as with the trains) of burst-patterned stimulation with 0·5 s long bursts at 20 Hz delivered once every 8 s (i.e. at an average frequency of 1·25 Hz). This protocol was completed by 10–15 min stimulation at 0·2 Hz to allow for recovery. Facilitating and depressing inputs

were identified by their changes in EPSP amplitude when stimulus frequency was increased from 0·2 to 1·25 Hz at the start of the tests.

Facilitating inputs. In five facilitating inputs tonic stimulation at 1·25 Hz increased quantal content of EPSPs relative to control stimulation at 0·2 Hz, reaching a plateau at $1{\cdot}11 \pm 0{\cdot}02$ times control within 10–30 s. With burst-patterned stimulation, the amplitudes of all EPSPs were further increased (Fig. 6*A*). This

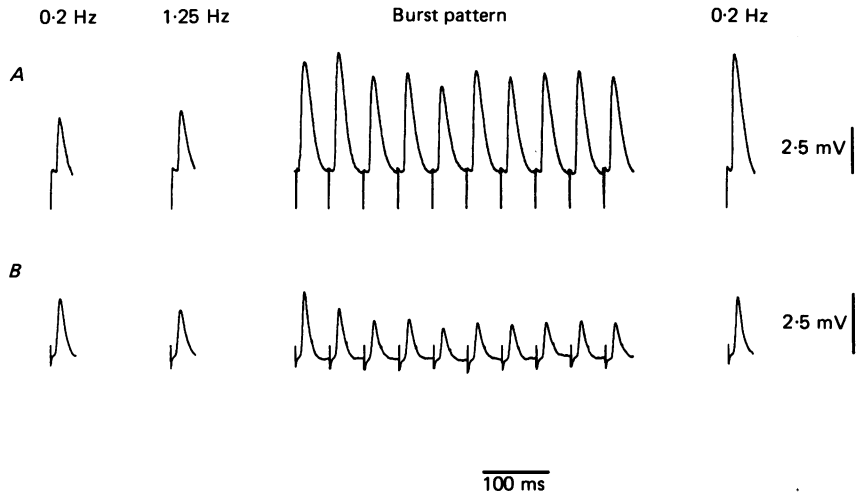

Fig. 6. Burst-patterned potentiation of a facilitating (*A*) and depressing (*B*) input compared to tonic stimulation at the same average frequency and to 0·2 Hz stimulation both before and after the test. Burst-patterned stimulation potentiated EPSP amplitude relative to tonic stimulation throughout the burst in the facilitating input but only the first two EPSPs in the burst in the depressing input. The order of stimulation is shown sequentially from left to right. Resumption of stimulation at 0·2 Hz following burst-patterned stimulation evoked EPSPs that remained potentiated relative to the 0·2 Hz control only in the facilitating input. The records are averages of twenty traces except for the last 0·2 Hz period where $N = 10$. Resting potential was -55 mV in *A* and -50 mV in *B*.

potentiation reached a maintained plateau within three to eight bursts (i.e. 16–56 s) when the average EPSP amplitude was $1{\cdot}66 \pm 0{\cdot}13$ times ($P < 0{\cdot}01$) that of EPSPs evoked by tonic stimulation at 1·25 Hz, and $1{\cdot}84 \pm 0{\cdot}14$ times the 0·2 Hz control. There was a decline in EPSP amplitude during the burst at the potentiated plateau in three of the experiments (Fig. 6*A*); in the other two it remained constant throughout. Thus in addition to the initial intraburst facilitation, a 0·5 s duration burst at 20 Hz activated a distinctly longer-lasting potentiation, with enhanced quantal output in every member of the burst after the first burst. In three further experiments it was found that when the interburst interval was increased from 8 to 16 s and the burst duration to 1 s (to keep the average frequency constant), the average burst potentiation was only slightly decreased from 1·66 to 1·51. Indeed, the burst potentiation persisted with little decay for nearly 1 min following the termination of stimulation (see below, also Fig. 6*A*).

A measure of the time course of burst-patterned potentiation was obtained from

TABLE 1. Changes in quantal content (m) and in the binomial parameters n and p during stimulation of facilitating inputs with trains and bursts

	Trains				Burst patterned			
	1·0–late / 0·2–late	5·0–late / 1·0–late	20–early / 1·0–late	20–late / 1·0–late	1·25 / 0·2	BP_{av} / 1·25	BP_1 / 1·25	BP_{8-10} / 1·25
No.	6	6	7	7	5	5	5	5
m	1·08 ± 0·04	1.26 ± 0·04	1·31 ± 0·10	0·96 ± 0·7	1·11 ± 0·02	1·66 ± 0·13	1·84 ± 0·05	1·52 ± 0·37
n	—	1·27 ± 0·09	1·25 ± 0·07	1·26 ± 0·11	—	—	1·38 ± 0·08	1·44 ± 0·28
p	—	1·01 ± 0·06	1·05 ± 0·04	0·79 ± 0·08	—	—	1·35 ± 0·07	1·13 ± 0·14

Column headings identify the test stimulation (Hz) over the control stimulation (Hz) to which measurements were normalized. 'Early' and 'late' have the same meaning as described in the legend to Fig. 2 and in more detail in the text. BP_{av} is the mean EPSP amplitude of all the members of the burst at the burst-patterned plateau. BP_1 and BP_{8-10} are measurements made at the burst-patterned plateau of the first and the last three EPSPs in the bursts, respectively. No. is the number of experiments. Values are presented as mean ± S.E.M. Note that the values of n were elevated in all the cases relative to the control, but there was no significant difference between them. By contrast values of the parameters m and p did differ significantly. These differences are summarized on Table 2.

TABLE 2. Significance of differences between the values of m and p listed in Table 1

Parameter m			Parameter p		
Comparison	Ratio	Significance	Comparison	Ratio	Significance
$BP_{av}/1{\cdot}25$	1·66	$P < 0{\cdot}01$	$\frac{BP_1}{1{\cdot}25} / \frac{5{\cdot}0}{1{\cdot}0}$	1·34	$P < 0{\cdot}005$
$\frac{BP_{av}}{1{\cdot}25} / \frac{5{\cdot}0}{1{\cdot}0}$	1·32	$P < 0{\cdot}01$	$\frac{BP_1}{1{\cdot}25} / \frac{20\text{–early}}{1{\cdot}0}$	1·29	$P < 0{\cdot}005$
$\frac{BP_{av}}{1{\cdot}25} / \frac{20\text{–early}}{1{\cdot}0}$	1·27	$P < 0{\cdot}05$	$\frac{BP_1}{1{\cdot}25} / \frac{20\text{–late}}{1{\cdot}0}$	1·71	$P < 0{\cdot}005$
$\frac{BP_{av}}{1{\cdot}25} / \frac{20\text{–late}}{1{\cdot}0}$	1·73	$P < 0{\cdot}001$	$\frac{BP_{8-10}}{1{\cdot}25} / \frac{20\text{–late}}{1{\cdot}0}$	1·43	$P < 0{\cdot}05$

Comparisons were made using paired or unpaired t tests where appropriate. Note that in all cases burst-patterned stimulation was significantly more effective than trains in increasing m and maintaining a high level of p.

the increase in the amplitude of the first EPSP in successive bursts. An example is shown in Fig. 7, in which a large potentiated plateau was reached in the first six to eight bursts. Potentiation was maintained for as long as burst-patterned stimulation was continued (maximum 11 min), and it took place without changes in MEPSP

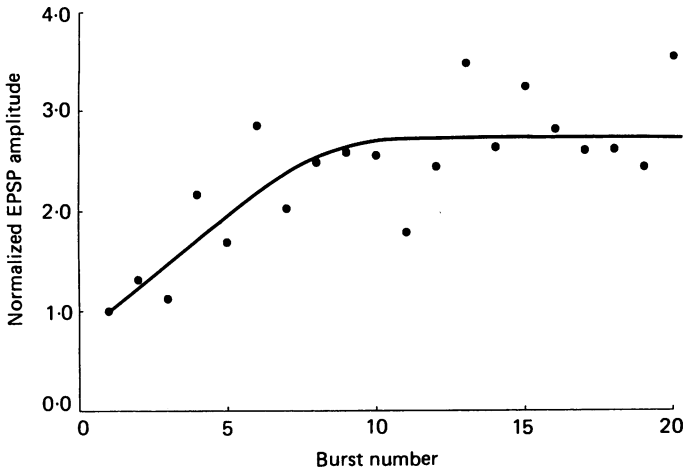

Fig. 7. The build-up of potentiation during burst-patterned stimulation of a facilitating input seen in the change in amplitude of the first EPSP in successive bursts. The potentiation reached a plateau during the first six to eight bursts. EPSP amplitude has been normalized relative to the amplitude of EPSPs evoked during preceding stimulation at 0·2 Hz. The curve was drawn by eye. Resting potential was −84 mV.

amplitude. The MEPSPs were measured as described earlier for the trains. Because of the lower frequency of MEPSPS relative to the 20 Hz trains MEPSPs occurring in the interburst interval and those occurring during the bursts were pooled. The MEPSP amplitude during burst-patterned stimulation was $1{\cdot}01 \pm 0{\cdot}10$ times that in tonic stimulation ($N = 5$). The potentiation was therefore due to an increase in quantal content. The maintained increase in quantal content was 1·32 ($P < 0{\cdot}01$) times greater than the maximum maintained facilitation observed with continuous trains (Table 2).

Binomial analysis of the burst-patterned responses was carried out on EPSPs sampled from the twenty to forty bursts at the potentiated plateau. The amplitude of the first EPSP of each of the bursts was taken and combined into one group, while the amplitudes of the last three EPSPs in the burst were combined into a second group. The burst-patterned potentiation of the first EPSP in the burst was associated with an increase of both n and p over the control values (Table 1). Whereas the increase of n with burst-patterned stimulation was not significantly different from the increase with trains, the burst-patterned potentiation of p in the beginning of the burst was significantly greater ($P < 0{\cdot}005$) than the facilitation of p during 5 and 20 Hz trains (Table 2). The value of p at the end of the burst declined; nevertheless it remained significantly ($P < 0{\cdot}05$) greater than late in the 20 Hz trains (Table 2). These results suggest that the main difference between repeated bursts and trains is the better maintenance of p during high-frequency activity. The

maintenance of the high value of p during the bursts may be related to their short duration and to the interval between them.

Depressing inputs. In nine depressing inputs (EPSP amplitude at 1·25 Hz was $0{\cdot}79 \pm 0{\cdot}04$ times the 0·2 Hz control), burst-patterned stimulation produced either a small potentiation or else a potentiation only of EPSPs early in the burst. Due to a substantial depression as the burst progressed, the average burst EPSP was only $1{\cdot}06 \pm 0{\cdot}04$ times larger than the tonic EPSP (Fig. 6*B*).

Effects of burst-patterned stimulation in non-perfused ganglia

Local accumulation of K^+ at synaptic sites reduces the amplitude of EPSPs (Erulkar & Weight, 1977) and in the environment of axons slows conduction (Hatt & Smith, 1976; Parnas, Hochstein & Parnas, 1976). We found similar effects when the ganglia were desheathed and superfused, rather than perfused.

During burst-patterned stimulation the first EPSP of the bursts was potentiated to $1{\cdot}55 \pm 0{\cdot}12$ ($N = 12$) times the value at 1·25 Hz. However, in most cases the amplitudes of the remaining EPSPs were progressively and profoundly depressed such that the average EPSP amplitude was not significantly greater ($1{\cdot}14 \pm 0{\cdot}07$) than the EPSPs evoked by tonic stimulation. This depression was accompanied by an increase in EPSP latency during the burst of $0{\cdot}38 \pm 0{\cdot}1$ ms by the tenth pulse which was significantly larger ($P < 0{\cdot}01$) than an increase of $0{\cdot}11 \pm 0{\cdot}04$ ms observed in perfused ganglia.

Because the stimulation was subthreshold for firing the impaled cells the major site of accelerated K^+ efflux must have been from the active preganglionic fibres. The results are therefore most easily accounted for by a local accumulation of K^+ at and close to the synaptic sites. The axons and the fine dendrites with which they make synaptic contact are located in the deep neuropil (Ramon y Cajal, 1952) where as many as ten to twelve fine processes are engulfed by single glial cells with only narrow mesaxonal connections to the bulk of the extracellular fluid (Birks, 1962). If these narrow clefts restrict diffusion of electrolytes and other solutes to and away from the fine neuronal processes at synaptic sites in the non-perfused ganglion, as our results suggest (see Fig. 1 and Methods), then they would be particularly susceptible to accumulation of extracellular K^+ as the above results indicate.

After-effects of burst-patterned stimulation

The amplitude of EPSPs following resumption of 0·2 Hz stimulation at the termination of 3 min burst-patterned activity in facilitating inputs remained potentiated for several minutes before returning to control levels (Fig. 8). During the first ten responses at 0·2 Hz, i.e. the first 50 s post-burst period, the fluctuations in EPSP amplitude were much smaller than later in the 4 min period of recovery, and the EPSPs tended to remain at the maximum potentiated level. An estimate of the maximum post-burst potentiation was made by pooling the average of the amplitudes of the first ten responses in the five facilitating inputs studied; the value was $1{\cdot}81 \pm 0{\cdot}25$ times the 0·2 Hz control. This compares with the average potentiation of all ten members of the bursts at plateau during burst-patterned activity of 1·84 times the same 0·2 Hz control. It therefore appears that the potentiation of EPSP amplitude during burst patterning persists with little decline for over 30 s.

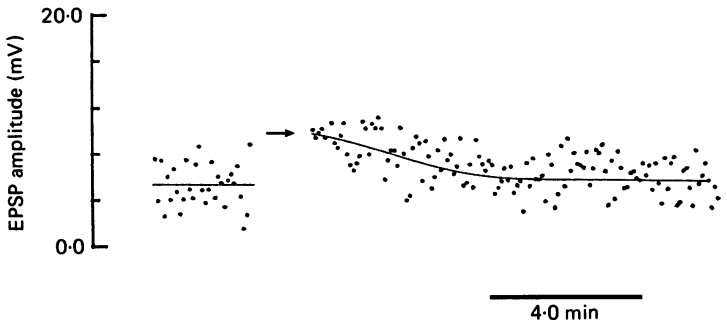

Fig. 8. Post-burst-patterned potentiation in a facilitating input following burst-patterned stimulation. EPSPs evoked at 0·2 Hz before and after a 3 min bout of burst-patterned stimulation (arrow). The arrow indicates the level of potentiation achieved at plateau during burst-patterned stimulation. Burst-patterned stimulation produced a potentiation which lasted for several minutes following the resumption of stimulation at 0·2 Hz. The initial portion of the post-burst-patterned potentiation was characterized by a decrease in EPSP amplitude fluctuation. Resting potential was −55 mV. Note that the level of potentiation achieved during the stimulation was maintained for over 30 s in the post-burst-patterned period before declining.

Post-tetanic potentiation following trains of impulses is known to be associated with increases in the binomial parameter p and also to depend on accumulation of Ca^{2+} during the trains (Rosenthal, 1969). It was accordingly of interest to examine the effects of lowered Ca^{2+} not only on the post-tetanic potentiation following burst-patterned stimulation but also on the growth of the potentiation during burst-patterned activity.

Effects of low Ca^{2+}

In eight experiments Ca^{2+} in the perfusing solution was reduced from 2·2 to 0·5 mM and either 1·2 mM-Mn^{2+} was added or Mg^{2+} was increased from1·2 to 5·0 mM. EPSP amplitude was reduced to between 1/15 and 1/40 of control under these conditions. In low Ca^{2+} all eight inputs examined showed a facilitation at 1·25 Hz to 1·31 $\pm$ 0·05 times the 0·2 Hz control. In low Ca^{2+} there was a pronounced facilitation during each burst (Fig. 9). This intraburst facilitation reached a maximum value between the fifth and seventh EPSP of the burst. Both facilitation at 1·25 Hz and during the bursts was considerably greater than that found in normal Ca^{2+}, as expected from previous work on guinea-pig SCG (McLachlan, 1975*a*). The average amplitude of all the EPSPs in the burst was 2·00 $\pm$ 0·21 times greater than that of the tonic EPSPs, late in the 1·25 Hz trains. However, the increase in EPSP amplitude during the bursts in low Ca^{2+} decayed completely in the interburst interval so that it did not summate in successive bursts. Thus in low Ca^{2+} although facilitation was larger there was no progressive interburst potentiation.

Post-burst-patterned potentiation was determined by taking the average of the amplitudes of the first ten responses at 0·2 Hz following termination of the 3 min period of burst-patterned activity. In low Ca^{2+} the post-burst-patterned potentiation was abolished, and the mean EPSP amplitude was 1·03 $\pm$ 0·06 times the control

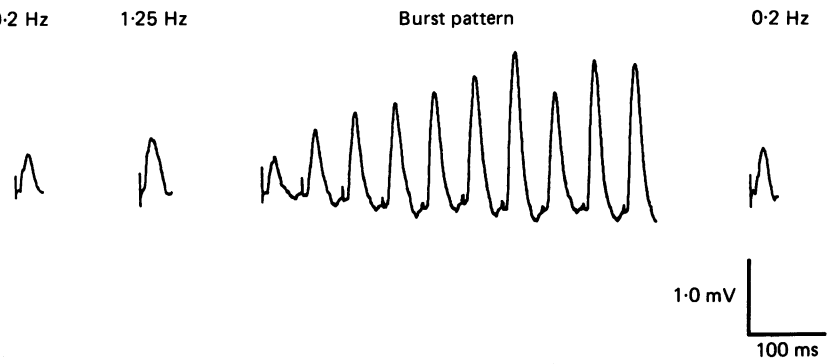

Fig. 9. Effects of tonic and burst-patterned stimulation in low Ca^{2+}. Ca^{2+} was reduced from 2·2 to 0·5 mM and Mg^{2+} raised from 1·2 to 5 mM. The EPSP amplitude in the beginning of the burst was not potentiated relative to the 0·2 Hz control. Burst-patterned stimulation in low Ca^{2+} produced no accumulated potentiation from burst to burst and maintained only the intraburst facilitation throughout the repetition of bursts. The order of stimulation is shown sequentially from left to right. Resumption of stimulation at 0·2 Hz evoked EPSPs that were only slightly potentiated relative to the 0·2 Hz control. Records are averages of twenty traces, except for the last 0·2 Hz period where $N = 10$. Resting membrane potential was −50 mV.

0·2 Hz value. Taken together with the fact that the interburst potentiation was also abolished in low Ca^{2+} these results suggest that the build-up and maintenance of burst potentiation and the generation of post-tetanic potentiation are linked by a common mechanism.

DISCUSSION

The main findings reported in this paper depended critically on two technical innovations. The first was the successful perfusion of isolated ganglia which greatly increased their viability during repetitive activation compared to the superfused desheathed preparation. This can be attributed to more rapid removal of K^+ from synaptic sites. The second was the ability to identify facilitating and depressing inputs well before the start of the experimental tests by the simple expedient of monitoring the change in EPSP amplitude on switching from 0·2 to 1·0 or 1·25 Hz stimulation. This permitted classification of subsequent responses to various forms of test stimulation as characteristic for each type of input.

Both facilitating and depressing inputs have been observed in guinea-pig SCG by Saachi & Perri (1971). In the present study the more complete analysis revealed that about half of the inputs were depressing, EPSP amplitude in these inputs declined as train frequency was increased, and they displayed little or no potentiation during or following burst-patterned stimulation. This failure of depressing inputs to increase EPSP amplitude in response to repetitive activity was associated with a reduction in the binomial parameter n as opposed to the increase found in facilitating inputs. There was little change in p in either type of input except late in 20 Hz trains where p decreased in both types of input.

It is striking that the fraction of preganglionic neurones that receive an input from the respiratory drive and therefore carry the burst-patterned form of activity has

been estimated in cats to be 40–50% (Preiss *et al.* 1975). It is reasonable to suppose that these neurones provide facilitating inputs to the postganglionic cells; but whether all post-ganglionic cells receive facilitating inputs is an important question not answered by the present investigation, although in a few cells both types of input were identified.

With the improvements in technique it was possible to demonstrate that in facilitating inputs the burst-patterned form of input produces a progressive increase in EPSP quantal content to a maintained plateau in which the average amplitude of EPSPs is 1·66 times greater than the average achieved during continuously stimulation at the same average frequency of 1·25 Hz.

What is of particular interest for the understanding of the processes underlying burst-patterned potentiation is that it was found to induce a long-lasting potentiation which persisted throughout the stimulation and for some 30–40 s after the end of stimulation before declining over the next 3 min. This entire process was abolished in low Ca^{2+}. In addition the binomial analysis revealed that while n was increased in both trains and burst-patterned stimulation, a major difference between these two forms of stimulation was the increase of p, particularly at the onset of the bursts at plateau. Thus the effect of low Ca^{2+} and the high level of p, both of which are prominent features of post-tetanic potentiation (Rosenthal, 1969), are also characteristic of burst-patterned potentiation. It is reasonable to suggest that during burst-patterned activity each burst is followed by a potentiation which lasts throughout the interburst interval and the next burst and sums with successive bursts to reach a plateau which is maintained.

This work establishes that the burst-patterned form of activity potentiates nicotinic transmission by a presynaptic action, and that the increase is greater than can be achieved with continuous trains at any frequency containing about the same number of pulses as the burst-patterned form. That is to say, at an average frequency of only 1·25 Hz the increase in quantal content by burst-patterned stimulation was greater than could be achieved by increasing train frequency even to 20 Hz. It may be concluded that the burst-patterned form of input is the most potent form of activity in promoting nicotinic transmision.

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REFERENCES

ANDERSSON, P. O., BLOOM, S. R., EDWARDS, A. V. & JARHULT, J. (1982). Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *Journal of Physiology* **322**, 469–483.

BACHOO, M. & POLOSA, C. (1987). Properties of the inspiratory-related activity of sympathetic preganglionic neurones of the cervical trunk in the cat. *Journal of Physiology* **385**, 545–565.

BENNETT, M. R., FLORIN, T. & PETTIGREW, A. G. (1976). The effect of calcium ions on the binomial statistic parameters which control acetylcholine release at preganglionic nerve terminals. *Journal of Physiology* **257**, 597–620.

BICHNELL, R. J., FLINT, A. P. F., LENG, G. & SHELDRICK, E. L. (1982). Phasic pattern of electrical stimulation enhances oxytocin secretion from the isolated neurohypophysis. *Neuroscience Letters* **30**, 47–50.

BIRKS, R. I. (1962). The effects of a cardiac glycoside on subcellular structuring within nerve cells and their processes in sympathetic ganglia and skeletal muscle. *Canadian Journal of Biochemistry and Physiology* **40**, 303–315.

BIRKS, R. I. (1978). Regulation by patterned preganglionic neural activity of transmitter stores in a sympathetic ganglion. *Journal of Physiology* **280**, 559–572.

BIRKS, R. I. (1979). Modulation of transmitter turnover by sodium and the sodium pump. *Journal of Physiology* **295**, 51–52*P*.

BIRKS, R. I. (1982). Acetylcholine release during burst-patterned stimulation of a sympathetic ganglion: its relation to transmission efficiency. *Journal de physiologie* **78**, 417–419.

BIRKS, R. I., LASKEY, W. & POLOSA, C. (1981). The effect of burst patterning of preganglionic input on the efficacy of transmission at the cat stellate ganglion. *Journal of Physiology* **318**, 531–539.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963). Spontaneous synaptic activity in sympathetic ganglion cells of the frog. *Journal of Physiology* **167**, 389–401.

BLISS, T. V. P. & LOMO, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**, 331–356.

BLOOM, S. R., EDWARDS, A. V. & GHATEI, M. A. (1984). Neuroendocrine responses to stimulation of the splanchnic nerves in bursts in the conscious adrenalectomized calf. *Journal of Physiology* **346**, 519–531.

BRIGGS, C. A., BROWN, T. H. & MCAFEE, D. A. (1985). Neurophysiology and pharmacology of long-term potentiation in the rat sympathetic ganglion. *Journal of Physiology* **359**, 503–521.

BRIMBLE, M. J., WALLIS, D. I. & WOODWARD, B. (1972). Facilitation and inhibition of cell groups within the superior cervical ganglion of the rabbit. *Journal of Physiology* **226**, 629–652.

BROWN, T. H. & SELYANKO, A. A. (1985). Membrane currents underlying the cholinergic slow excitatory post-synaptic potential in the rat sympathetic ganglion. *Journal of Physiology* **365**, 365–387.

DEL CASTILLO, J. & KATZ, B. (1954). Statistical factors involved in neuromuscular facilitation and depression. *Journal of Physiology* **124**, 574–585.

DUNANT, V. & DOLIVO, M. (1968). Plasticity of synaptic functions in the excised sympathetic ganglion of the rat. *Brain Research* **10**, 272–273.

DUTTON, A. & DYBALL, R. E. (1979). Phasic firing enhances vasopressin release from the rat neurohypophysis. *Journal of Physiology* **290**, 433–440.

EDWARDS, A. V. (1982). Adrenal catecholamine output in response to stimulation of the splanchnic nerve in bursts in the conscious calf. *Journal of Physiology* **327**, 409–419.

ERULKAR, S. D. & WEIGHT, F. F. (1977). Extracellular potassium release and transmitter release at the giant synapse of squid. *Journal of Physiology* **266**, 209–218.

GALVAN, M., TEN BRUGGENCATE, G. & SENEKOWITSCH, R. (1979). The effects of neuronal stimulation and ouabain upon extracellular K^+ and Ca^{2+} levels in rat isolated sympathetic ganglia. *Brain Research* **160**, 544–548.

GRUNDY, D. & SCRATCHERD, T. (1982). Effects of stimulation of the vagus nerve in bursts on gastric acid secretion and motility in the anaesthetized ferret. *Journal of Physiology* **333**, 451–461.

HATT, H. & SMITH, D. O. (1976). Synaptic depression related to presynaptic axon conduction block. *Journal of Physiology* **259**, 367–393.

KUNO, M. (1964). Mechanisms of facilitation and depression of the excitatory synaptic potential in spinal motoneurones. *Journal of Physiology* **175**, 100–112.

LARABEE, M. G. & BRONK, D. W. (1947). Prolonged facilitation of synaptic excitation in sympathetic ganglia. *Journal of Neurophysiology* **10**, 139–154.

LILEY, A. W. (1956). The quantal components of the mammalian end-plate potential. *Journal of Physiology* **133**, 571–587.

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

MCLACHLAN, E. M. (1975*b*). Changes in statistical release parameters during prolonged stimulation of preganglionic nerve terminals. *Journal of Physiology* **253**, 477–491.

MCLACHLAN, E. M. (1978). The statistics of transmitter release at chemical synapses. *International Review of Physiology, Neurophysiology III* **17**, 49–117.

MARTIN, A. R. & PILAR, G. (1964). Presynaptic and post-synaptic events during post-tetanic potentiation and facilitation in the avian ciliary ganglion. *Journal of Physiology* **175**, 17–30.

PARNAS, I., HOCHSTEIN, S. & PARNAS, H. (1976). Theoretical analysis of parameters leading to frequency modulation along an inhomogeneous axon. *Journal of Neurophysiology* **4**, 909–923.

PREISS, G., KIRCHNER, F. & POLOSA, C. (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. *Brain Research* **87**, 363–374.

RAMON Y CAJAL, S. (1952). *Histologie du systèm nerveux de l'homme et des vertebres*. Translated by L. AZONLAY. Madrid: Consejo Superior de Investigaciones Científicos.

ROSENTHAL, J. (1969). Post-tetanic potentiation at the neuromuscular junction of the frog. *Journal of Physiology* **203**, 121–133.

SACCHI, O. & PERRI, V.(1971). Quantal release of acetylcholine from the nerve endings of the guinea-pig superior cervical ganglion. *Pflügers Archiv* **329**, 207–219.

THIES, R. E. (1965). Neuromuscular depression and the apparent depletion of transmitter in mammalian muscle. *Journal of Neurophysiology* **28**, 427–442.