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On the Contribution of Quantal Secretion from Close-Contact and Loose-Contact Varicosities to the Synaptic Potentials in the Vas Deferens

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On the contribution of quantal secretion from close-contact and loose-contact varicosities to the synaptic potentials in the vas deferens

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SUMMARY

A bidomain model of the smooth muscle syncytium has been used to analyse the sources of transmitter secretion that give rise to the excitatory junction potential (EJP) in the guinea-pig vas deferens. The timecourse of the spontaneous excitatory junction potential (SEJP) has been taken to be the same as the time course of action of a quantum of transmitter. The amplitude of the SEJP is dependent on both the size of the quantum secreted and the distance away of the source of the quantum from the muscle cells. Two such sources are considered, one identified as the close-contact varicosities (CCVs) about 50 nm from the muscle and the other as loose-contact varicosities (LCVs) at greater distances. It is shown that in order for the syncytium to reach equipotential by the time the EJP has declined to about 80% of its peak, each muscle cell must receive a quantum of transmitter. The relatively low density of innervation of muscle cells by CCVs so far reported, together with the extremely low probability for secretion from these, indicates that many LCVs surrounding each muscle cell contribute to the EJP.

The rising phase of the EJP contains components that indicate the sources of the transmitter responsible for its generation, and these components have been made explicit by differentiating the EJP to give the DEJP. This always has a smooth and relatively slow component that lasts for about 80 to 100 ms and

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occasionally has fast components superimposed on it. These latter are shown to be almost certainly due to secretion of quanta from the CCVs. It is known that there is a distribution of action potential velocities in the sympathetic nerves to the vas deferens. To account for this, the secretion of quanta from different CCVs on a set of muscle cells in the syncytium were given different delays so that the DEJP consisted of a slow wave form that extended over 80 ms, composed of clearly discernible components arising from the CCVs. This wave form could be smoothed by allowing each cell in the syncytium to receive a quantum of transmitter from a CCV, a condition that did not then allow for the appearance of fast components in the DEJP.

A model that generated both the non-intermittent slow component of the DEJP and the intermittent fast component consisted of each cell in the syncytium receiving an innervation from a single CCV as well as from a large number of LCVs. In this case, all the varicosities could secrete a quantum of transmitter with a particular probability after a delay characteristic for that varicosity. The size of the quanta were drawn from distributions with means that were graded according to the distance of the varicosities from the muscle cells. It is shown that under these conditions the model can account for the observed combinations of fast and slow components of the DEJP. Also accounted for are the effects of stimulating the intramural sympathetic nerves compared with stimulating the hypogastric nerve, as well as the effects of increasing the number of nerves stimulated and increasing the calcium concentration. The suggestion is made that the EJP is due to the LCVs in this preparation with the occasional secretion from a CCV modifying the rate of rise of the EJP.

1. INTRODUCTION

The electrophysiological analysis of autonomic neuromuscular transmission with intracellular electrodes was begun by Burnstock & Holman (1961), who showed that in the guinea-pig vas deferens the excitatory junction potential (EJP) lasts for several hundred milliseconds whereas the spontaneous excitatory junction potential (SEJP) is of much briefer duration (Burnstock & Holman 1962). Attempts were subsequently made to determine if the declining phase of the EJP could simply be considered in terms of the discharge of the membrane capacitance following the charging of this during the period of membrane conductance change due to the action of the transmitter (Fatt & Katz 1951). Estimates of the time constant of the membrane with extracellular electrodes gave values of about 100 ms (Tomita 1967), which is considerably less than the time constant of decline of the EJP of between 160 and 450 ms (Bennett 1972; Cunnane & Manchanda 1989). This led to the idea that the EJP might be due to the secretion of transmitter from varicosities throughout a smooth muscle bundle (Bennett 1972). If transmitter secreted from all the varicosities in a smooth muscle bundle contributes to the conductance change responsible for the EJP, then the timecourse of diffusion of the transmitter out of the muscle bundle may be sufficiently long to explain the long time of the declining phase of the EJP (Bennett 1972, 1973).

This problem concerning the time course of decline of the EJP was resolved by subsequent determinations of the time constant of the membrane. Bywater & Taylor (1980) discovered that another interpretation of the experimental results of Tomita (1967) was possible if allowance was made for the finite distance between the stimulating electrodes (Davis & Lorente de Nó 1947). Application of the Hodgkin & Rushton (1946) cable equations to the electrotonic spread in the muscle, when allowance was made for this, gave a time constant for the membrane which was similar to the time constant of decline of the EJP from about 80% of its peak (Bywater & Taylor 1980). The declining phase of the EJP appears then to be due to the passive discharge of the membrane capacitance after the syncytium reaches equipotential at about 80% of its peak value.

The SEJP declines exponentially with a time constant of about 30 ms (Bennett 1972; Cunnane & Manchanda 1989), which is much faster than the membrane time constant of about 210 ms determined by Cunnane & Manchanda (1990). The response of single cells in the smooth muscle syncytium to current injection occurs in a few milliseconds (Bennett 1967), so that the timecourse of the SEJP must be similar to the timecourse of the underlying conductance change due to the quantum of transmitter (figure 2*a*). These very fast time constants for intracellular current injection in the smooth muscle arise because of the syncytial structure of the tissue, in which current injection at a point can escape in three dimensions into surrounding cells (Bennett 1972). In the original description of autonomic neuromuscular transmission the EJP was shown to reach its peak value in about 100 ms, but occasionally cells were impaled that possessed EJPs with very fast components on the rising phase that resembled the SEJP (see figure 7 in Burnstock & Holman, 1962). Blakeley & Cunnane (1979) differentiated the rising phase of the EJP in the guinea-pig vas deferens (giving the DEJP) to give a more careful quantitative account of this, and enable the fast components to be separated from the slow ones. Their results showed that the DEJP consisted of both intermittently occurring fast components, with time courses similar to that of the differential of the SEJP, as well as a consistently appearing slow component that lasted for about 80 to 100 ms (figure 1).

This analysis has been repeated many times (see, for example, Cunnane & Stjärne 1984; Stjärne & Åstrand 1984), with the general interpretation that the fast component of the DEJP is due to secretion from close-

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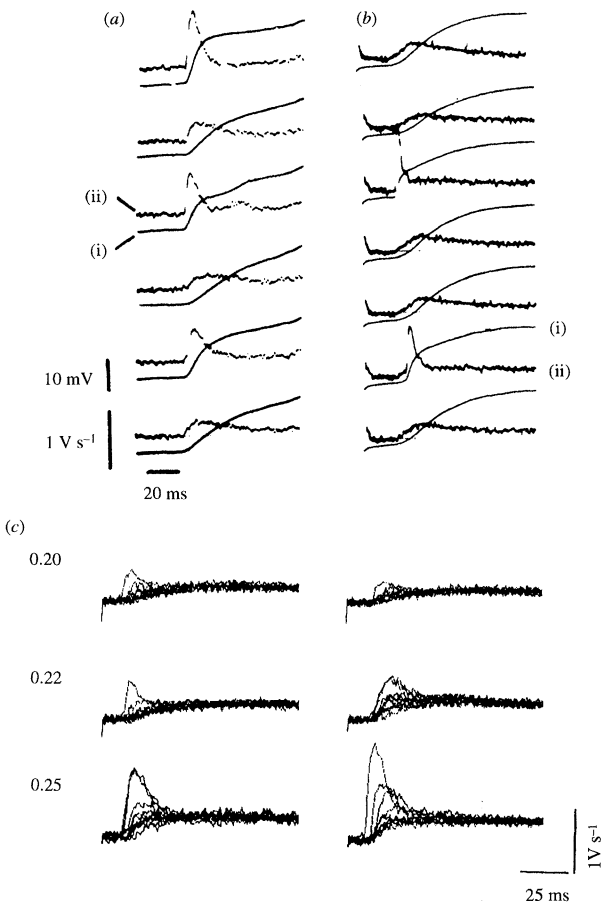

Figure 1. (*a*) and (*b*) show intracellular records of the rising phase of the EJP in two different smooth muscle cells in the guinea-pig vas deferens (noiseless trace, i) as well as the differential of the EJP (dV/dt, noisy trace, (ii)). The EJPs were evoked by trains of submaximal stimuli (0.91 Hz) to the postganglionic hypogastric nerves and successive records of the EJP are shown after facilitation was complete. (Records are from fig. 1 (II) and fig. 6 (A) of Blakeley & Cunnane (1979); reproduced with permission from *The Journal of Physiology*.) (*c*) shows just the time-derivative of the EJP rising phase for a single smooth muscle cell in the guinea-pig vas deferens. The numbers on the left refer to the increase in duration of the stimulating pulse to the intramural nerves, delivered at 1 Hz; several records have been taken at each stimulus strength. (Records are from Fig. 3 (A) in Cunnane & Stjärne (1984); reproduced with permission from *Neuroscience*.) All other figures in this paper show simulated responses.

contact varicosities (CCVs) within about 50 nm of the cell recorded from; the slow component has been taken as due to the radiation, into the cell recorded from, of the electrical results of transmitter secreted onto surrounding cells from their CCVs. It seems likely that both the slow and fast components of the EJP are due to the secretion of ATP rather than noradrenaline. The EJP is reversibly abolished by α,β-methylene ATP, the enzymically stable, desensitizing analogue of ATP (Cunnane & Manchanda 1988; Brock & Cunnane 1988). Furthermore, applied ATP but not noradrenaline can evoke depolarization in the guinea-pig vas deferens which closely resembles the EJP (Sneddon & Westfall 1989; Cunnane & Manchanda 1988, 1990). Finally, although the drug suramin blocks over 90% of the EJP in the guinea-pig vas deferens (Sneddon 1992), and this has been taken as evidence in favour of ATP acting as the transmitter, it is possible that a small component of the EJP could be due to other purine transmitters (von Kügelgen & Starke 1991).

The idea of CCVs is due to Dale (1935), who hypothesized their existence to explain the apparent inability of certain blocking drugs to gain access to the receptors at particularly close regions of apposition between nerve terminals and muscle cells. Such CCVs are now defined as approaches of varicosities to within about 50 nm of a smooth muscle cell in which the basal laminae of the varicosity and the muscle cell fuse (Richardson 1962; Merrillees *et al.* 1963). Although it has been estimated that every smooth muscle cell in the mouse vas deferens may possess a CCV (Burnstock & Iwayama 1971), a much lower density of these varicosities has been reported for the guinea pig vas deferens, although only the varicosities occurring at axon terminations were considered (Bennett & Merrillees 1966; Merrillees 1968). It is likely, however, that both studies on the mouse and guinea-pig vas deferens underestimated the number of CCVs due to failure to faithfully reconstruct individual varicosities with serial sections at the ultrastructural level (Luff & McLachlan 1988).

Models of the three-dimensional smooth muscle syncytium (Bennett 1973; Purves 1976) allow for an analysis of various hypotheses regarding the origin of the fast and slow components of the DEJP. In this work, advantage has been taken of a recent extension of these models (Bennett *et al.* 1993) which allows for estimates to be made of both the extracellular current flow and the transmembrane potentials in the syncytium during synaptic transmission, in order to try and identify the likely sources of transmitter secretion that give rise to the EJP.

2. THEORY

Previously (Bennett 1972, 1973; Purves 1976) the smooth muscle bundle has been modelled as a three-dimensional rectangular grid, the nodes representing the muscle cells and the joining lines representing the intracellular resistances. To include the interstitial medium, it is necessary to extend this to a bidomain model, in which there are now two grid systems, one representing the intracellular region and the other the interstitial region. These two grids occupy the same region of space, and are connected at every node by an RC circuit representing the membrane of a muscle cell. The muscle tissue occupies the region $z \leqslant 0$, and the nodes of the grid are labelled by the rectangular cartesian coordinates (i,j,k) where i,j,k take integer values with $-\infty < i < \infty$, $-\infty < j < \infty$, $-\infty < k \leqslant 0$.

The details of this approach are contained in Bennett *et al.* (1993), where a method is given for solving for the excess membrane potential (the EJP) and the current measured by a surface electrode (the EJC). The DEJP is then found by numerical differentiation of the EJP. A summary of the theory is given in the Appendix.

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Table 1. *Values of the parameters used in the numerical calculations*

(The last three quantities are defined in terms of the earlier ones, so their numerical values follow from the ones already assigned. The assumed grid spacings are 4 μm in the x- and z-directions and 140 μm in the y-direction. The value of τ_g was 14.3 ms in all cases except that of figure 10, in which case it was 33.3 ms. The values of g_0 ranged from 0.75 nS to 65 nS and these are given in the legends for each particular case. The values given for the 'space constant' are multiples of the grid spacing in the corresponding directions. In each case considered, τ_g had the same value for both CCVs and LCVs, except figure 10 (see legend).)

quantity	symbol	value
membrane resistance	R_m	$3.6 \times 10^9 \Omega$
membrane capacitance	C_m	5.6×10^{-11}F
intracellular resistance	$\mathbf{R}_i$	$(14, 1750, 14) \times 10^6 \Omega$
extracellular resistance	$\mathbf{R}_e$	$(3.5, 437.5, 3.5) \times 10^6 \Omega$
driving potential	E_0	5×10^{-2}V
space constant	$\mathbf{\Lambda}$	(14.34, 1.28, 14.34)
membrane time constant	τ_m	0.2 s
anisotropy ratio	κ	0.25

The numerical values used for the parameters are the same as in Bennett *et al.* (1993), and are summarized in table 1.

3. FACTORS DETERMINING THE TIME OF DECLINE OF THE EJP

(*a*) *The effect of different distributions of close-contact varicosities*

Quantal secretion of transmitter from a CCV on a muscle cell in the syncytium has been represented in the present work by a conductance change in the muscle cell that follows an alpha function (Jack & Redman 1971; Jack *et al.* 1975). A value of $\tau_g =$ 14.3 ms in the expression for the conductance change in a single cell in the syncytium (Appendix, equation (2)) was found to give a timecourse for the SEJP that was similar to that observed in the guinea-pig vas deferens (figure 2*a*): the SEJP reaches its peak value in about 18 ms and then declines approximately exponentially with a time constant of 30 ms; the peak value of the SEJP is determined by the peak conductance and the value of the driving potential for transmission, which were taken as 50 nS and 50 mV respectively. If the muscle is given a very dense innervation in which each muscle cell receives a CCV, together with a very high probability for secretion, then the declining phase of the EJP is greatly extended (figures 2*b* and 3*c*). This occurs because all the cells reach equipotential after the EJP has declined to about 80 % of its peak value, so that most of its decline occurs with the time constant of the muscle cell membrane of 200 ms (figure 3*d*). It should

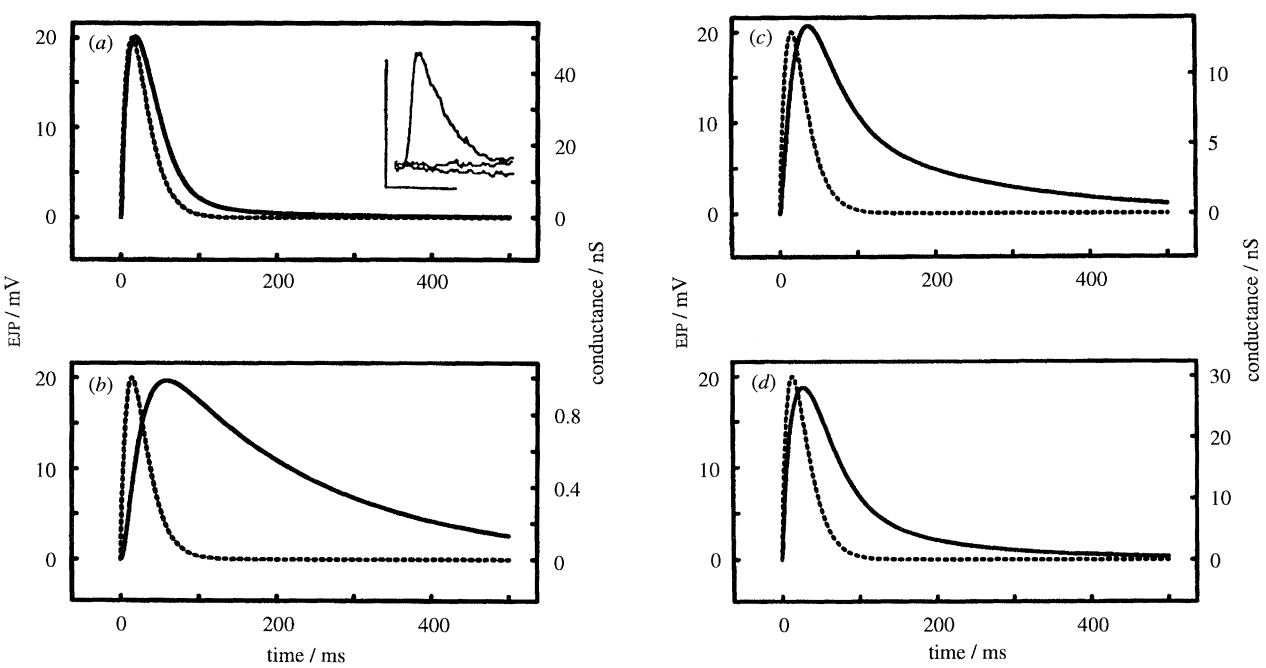

Figure 2. The effect of secretion from different numbers of close-contact varicosities on the timecourse of the EJP. The continuous line gives the EJP and the broken line gives the timecourse of the conductance change due to secretion. (*a*) The EJP in a cell due to secretion from a single varicosity on that cell. The insert shows the timecourse of an experimentally recorded spontaneous EJP (from figure 1*B* in Cunnane & Manchanda (1988); the calibration bars are 5 mV (vertical), 100 ms (horizontal). (*b*) The EJP in a cell due to secretion from varicosities placed on every cell in the smooth muscle syncytium. (*c*) The EJP in a cell due to secretion from varicosities placed on every 27th cell in the syncytium; the EJP is from one of the cells with a varicosity on it. (*d*) The EJP in a cell due to secretion from varicosities placed on every 216th cell in the syncytium; the EJP is from one of the cells that receives a direct innervation from a varicosity. The value of τ_g was 14.3 ms and the peak conductance (g_0) was 50, 1, 13 and 30 nS for cases (*a*), (*b*), (*c*) and (*d*) respectively. The cell recorded from in the syncytium was at the origin (0, 0, 0) of the rectangular coordinate system giving the positions of the cells. The different densities of close-contact varicosities were chosen to show the effect of these on the timecourse of decline of the EJP; the conductance values were chosen to give EJPs with about 20 mV peak amplitude.

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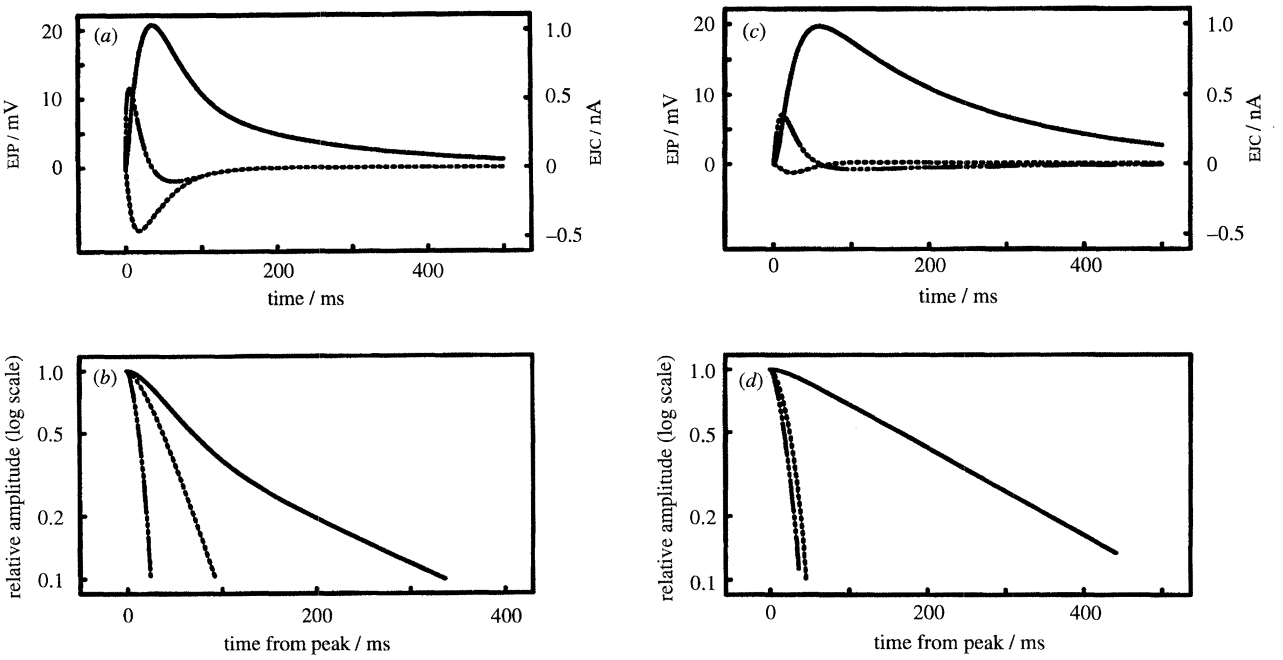

Figure 3. Timecourse of the EJP, the EJC, and the time derivative of the EJP (DEJP) due to secretion from varicosities onto one in 27 of the muscle cells in the syncytium, (*a*) and (*b*) or secretion from varicosities onto every muscle cell in the syncytium, (*c*) and (*d*). (*a*) Gives the EJP (continuous line), the EJC (broken line) and the DEJP (dot-dashed line). (No amplitude scale is shown for the DEJP; it peaks at 1.16 V s^{-1} at a time of 6 ms.) (*b*) Timecourse of the EJP, the EJC and the DEJP on log-linear coordinates to show the relative exponential character of the declining phase of these potentials and currents. Note that the EJP declines exponentially after reaching about 28 % of its peak value. (*c*) Gives the EJP, the EJC and the DEJP. (No amplitude scale is shown for the DEJP; it peaks at 0.71 V s^{-1} at a time of 12 ms.) (*d*) Timecourse of the EJP, the EJC and the DEJP on log-linear coordinates to show the relative exponential character of the declining phase of these potentials and currents. Note that the EJP declines exponentially after reaching about 80 % of its peak value. The value of τ_g was 14.3 ms and of g_0 was 13 nS. The cell recorded from was at the origin, and the electrode was 50 μm in diameter with its centre placed at the origin.

be noted that the peak conductance value due to a quantum of transmitter during the EJP had to be reduced to 2 % of that used for the secretion of a single quantum in the SEJP in order for them to produce the same peak depolarization (compare figure 2*b* with figure 2*a*). The result indicates that if only CCVs contribute to the EJP then either few muscle cells receive a CCV and/or they secrete with a low probability in the guinea-pig vas deferens (see also Purves 1976).

If muscle cells receive fewer CCVs, or if these secrete with low probability on arrival of the nerve impulse, then the EJP declines at first rapidly and then more slowly with the membrane time constant (figures 2*c* and 2*d*).

The rapid component occupies more of the declining phase of the EJP if fewer CCVs secrete (figures 2*c*,*d*). This occurs because the syncytium does not reach equipotential until later stages in the declining phase of the EJP (compare figure 3*b* with figure 3*d*), due to the individual cells discharging their membrane capacitance through the couplings between the cells rather than across the membrane resistance. With probabilities for secretion of at the most 0.03 in the guinea-pig vas deferens (Cunnane & Stjärne 1984; Brock & Cunnane 1988), at least 40 or so CCVs would have to innervate each cell for the syncytium to reach equipotential shortly after the peak of the EJP. As this seems unrealistic, other varicosities have been considered.

(*b*) *The effects of different distributions of loose-contact varicosities*

Bennett & Merrillees (1966) have described large numbers of loose-contact varicosities (LCVs) around each smooth muscle cell in the guinea-pig vas deferens, although this is yet to be confirmed by small section analysis through individual varicosities. As the time course of the conductance change due to the secretion of a quantum from a CCV (which is about 50 nm from a muscle cell) is relatively long (figure 2*a*), it is very likely that this is not limited by the times for diffusion of the transmitter from varicosity to receptors; rather, it is likely to be determined by the kinetics of interaction between transmitter and receptors (Cunnane & Manchanda 1989). The timecourse of action of quanta secreted from LCVs, up to say 200 nm from a muscle cell, is also unlikely to be determined by the diffusion times. It is not known if the diffusion times are different for CCVs and LCVs on surface smooth muscle cells from which the recordings in figure 1 were taken. The timecourse of the conductance change due to quanta from LCVs was therefore taken to be the same as that for the CCVs, that is $\tau_g = 14.3$ ms. However, the value of the peak conductance change was much less than that for CCVs: each cell was given 10 LCVs, with peak conductances drawn from a gamma distribution with mean 0.1 and variance 0.0033 (the gamma distribution, table 2, has parameter $r = 3$ in all the

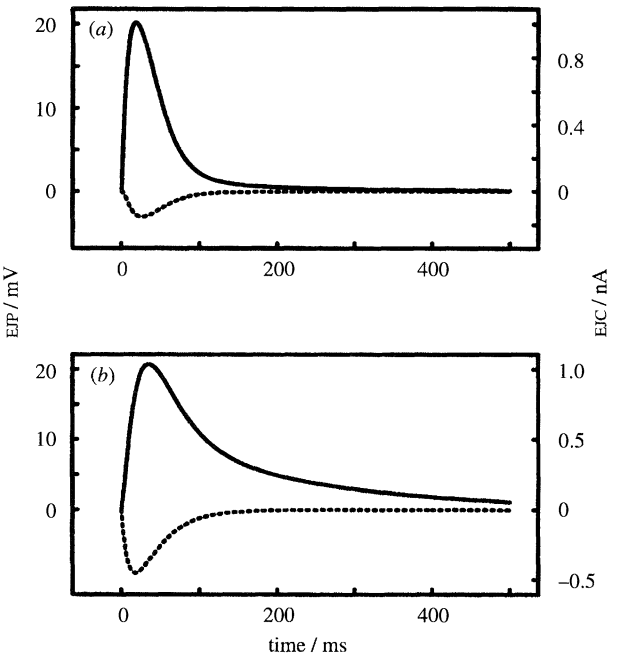

Figure 4. The current recorded with an extracellular electrode placed on a close-contact varicosity when secretion occurs from different numbers of close-contact varicosities throughout the syncytium. The continuous line gives the EJP and the broken line the time course of the extracellular current (EJC) recorded with the electrode. (*a*) The timecourse of the EJP and the EJC when secretion occurs from a single varicosity on that cell. (*b*) The timecourse of the EJP and the EJC in a cell due to secretion from varicosities placed on every 27th cell in the syncytium, including the one recorded from. The value of τ_g was 14.3 ms and of g_0 was 65 nS for (*a*) and 13 nS for (*b*). The cell recorded from was at the origin, and the electrode was 50 μm in diameter with its centre placed at the origin.

subsequent cases). Quantal secretion from these gave, as expected, a syncytium that reached equipotential just after the peak of the EJP and then declined exponentially with the membrane time constant. Although LCVs are likely to have a low probability for secretion, as do the CCVs, the very large number of the former compared with the latter makes it likely that each cell receives quanta secreted from several of these following a nerve impulse. These varicosities are therefore likely to be the main determinant of the syncytium reaching an equipotential just after the peak of the EJP.

(*c*) *Extracellular current flow during the decline of the EJP*

An extracellular electrode placed over the surface of a visualized varicosity can be used to record the current flow that results from a single quantal secretion from that varicosity (figure 4). In the case of spontaneous quantal secretion from a single varicosity, the extracellular current flow follows the same time-course as the underlying conductance change and the resultant SEJP (figure 4*a*). During evoked quantal secretion onto many cells in the syncytium (say, every 27th), the extracellular current flow is of shorter duration than the consequent membrane potential modulation (figure 4*b*). This occurs since when equipotential is reached in the syncytium there is no driving force for the current to flow between cells.

4. THE CONTRIBUTION OF CCVS TO THE RISE TIME OF THE EJP

(*a*) *The timecourse due to different distributions of CCVs*

The most sensitive measure introduced to ascertain the time course of the rising phase of the EJP is the DEJP. This also indicates the contributions of CCVs to the EJP, as illustrated in figure 5. Intracellular recording from a muscle cell that alone in the syncytium receives a quantal secretion with an underlying peak conductance of 20 nS gives an SEJP that peaks in about 19 ms and has a DEJP that peaks at 1.25 V s^{-1} in a few ms

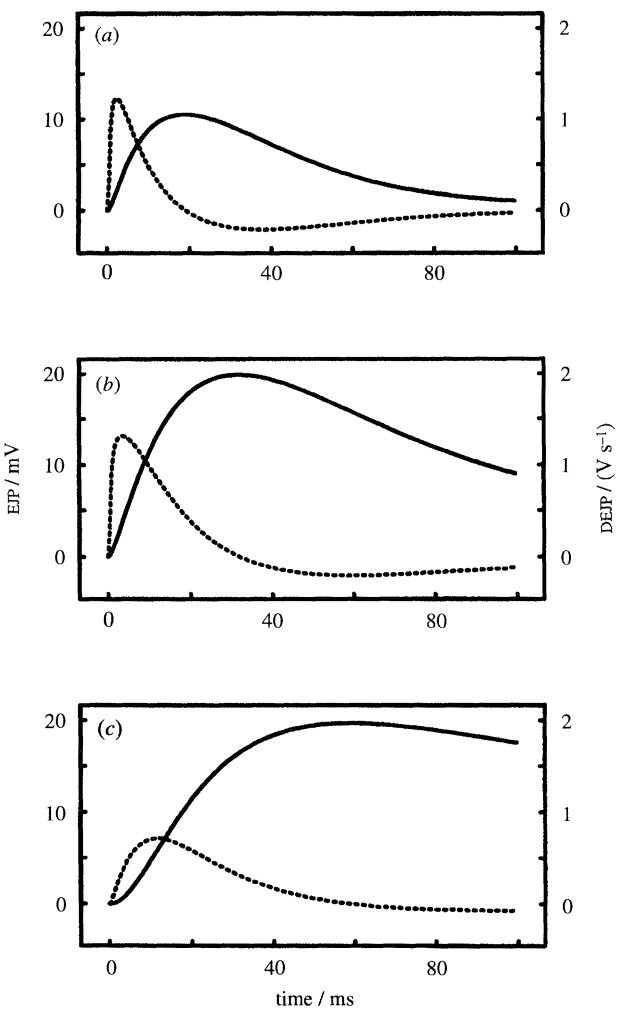

Figure 5. Time courses of the EJP and the DEJP due to the secretion of transmitter from different distributions of close-contact varicosities in the syncytium. The continuous line gives the EJP and the broken line the DEJP. (*a*) The result of secretion from a single close-contact varicosity in the syncytium; the recording is from the cell which received this innervation. (*b*) The result for secretion onto every 64th cell in the syncytium, including the cell recorded from. (*c*) The result for secretion onto every cell in the syncytium, including the cell recorded from. The value of τ_g was 14.3 ms in all cases and g_0 was equal to 20 nS in (*a*) and (*b*) and to 1 nS in (*c*). The cell recorded from was at (0, 0, 0).

(figure 5*a*); this compares favourably with the experimental results of figure 1 for the guinea-pig vas deferens. In contrast, if this same conductance change occurs in every 64th muscle cell in the syncytium during evoked secretion, as each of these receives a quantal secretion, then the EJP peaks in 32 ms and has a DEJP that peaks at 1.3 V s^{-1} in a few ms as before (figure 5*b*) although it now declines to zero in 32 ms rather than 19 ms as before. The DEJP clearly has a very different time course to that recorded, as there is no slow component lasting for about 100 ms, responsible for the rising phase of the EJP lasting for this time (compare figure 5 with figure 1).

A slow DEJP can be obtained if quantal secretion occurs on all the cells in the syncytium; this requires that the conductance change due to a quantal secretion be dropped from 20 nS to 1 nS otherwise the EJP will saturate at the equilibrium potential for transmission (see also Purves 1976). In this case, the EJP peaks at the later time of 60 ms and so the DEJP declines to zero at this time (figure 5*c*). There are three difficulties in attributing the time course of the rising phase of the EJP to this kind of innervation: first, it requires an exceptionally high innervation of muscle cells in the guinea-pig smooth muscle syncytium, each receiving a quantum of transmitter on arrival of the nerve impulse; second, the most common rise time for the EJP is 100 ms not 60 ms; third, and most important, there cannot be any fast DEJP component on the slow DEJP as all the CCVs are engaged in generating the slow DEJP and fast components do not then emerge.

(*b*) *The effect of conduction velocity dispersion to CCVs*

It has been shown experimentally that if the guinea-pig vas deferens is stimulated via the intramural nerves then the peak of the EJP occurs at about 60 ms. On the other hand, if the extrinsic postganglionic hypogastric nerve is stimulated the EJP peaks in about 100 ms (Blakeley & Cunnane 1979). Thus part of the time to the peak of the EJP due to hypogastric nerve stimulation (figure 1*a*,*b*) is due to conduction velocity dispersion. Therefore, in seeking a possible solution to the problem of obtaining fast component DEJPs on top of the slow DEJP during the rising phase of the EJP, consideration was given to the differences in conduction velocities of the axons that give rise to the CCVs. Conduction velocities were chosen so that they gave rise to an exponential distribution of delays in quantal secretion from the CCVs throughout the syncytium following a nerve impulse. If a quantum of transmitter is secreted onto every 64th cell in the syncytium, and this gives rise to a conductance change of 20 nS occurring with a delay drawn from a distribution with mean 30 ms and standard deviation 30 ms, then the EJPs are like those given in figure 6: the results are for three adjacent cells, one with a CCV and two without. The result of including the delays is to increase the time to peak of the EJP to over 60 ms for those cells that do not receive a direct input (figure 6*b*,*c*) so that the DEJP lasts for this time before it goes negative, although there are a number of bumps on the DEJP that are not observed on

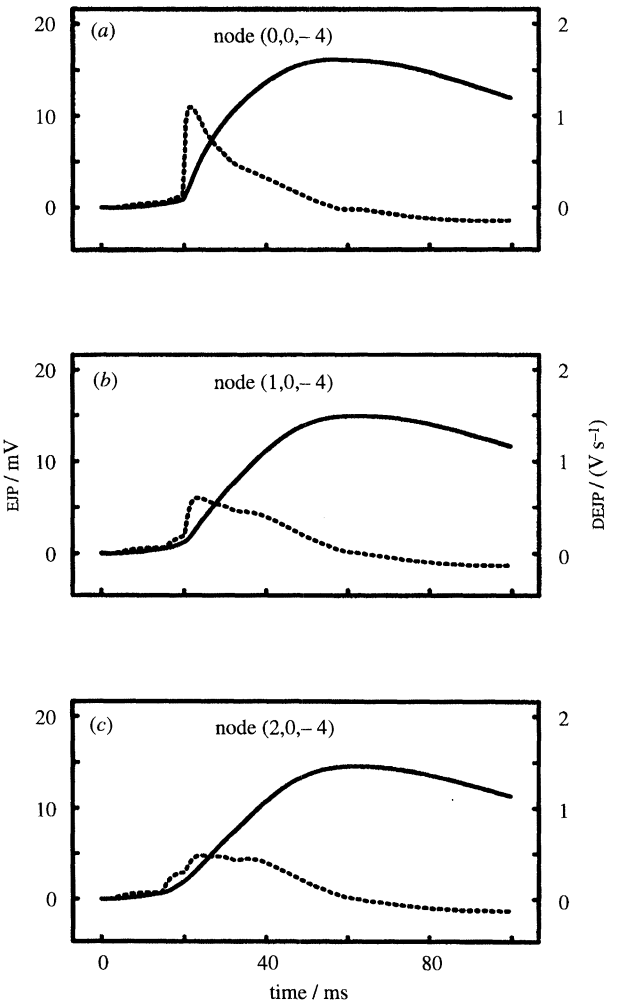

Figure 6. The effect of an exponential distribution of conduction velocities for the different axons giving rise to close-contact varicosities in the syncytium on the timecourse of the EJP and the DEJP recorded in different cells when the syncytium receives a sparse innervation. Every 64th cell in the syncytium was innervated by a close-contact varicosity. The continuous line gives the EJP and the broken line the DEJP. The results in (*a*), (*b*) and (*c*) are for cells at the positions $(0,0,-4)$, $(1,0,-4)$, $(2,0,-4)$ respectively. The values of τ_g and g_0 were 14.3 ms and 20 nS respectively. The exponential distribution of delays due to the different conduction velocities had a mean of 30 ms and a standard deviation of 30 ms.

the experimental DEJP. These bumps are due to the inputs from nearby cells that receive a direct quantal secretion. In one of these (figure 6*a*) the direct input gives rise to an EJP that peaks in 56 ms so the slow component of the DEJP is complete in this time; this occurs because there are few cells nearby that receive a quantal secretion.

To see if the distribution of delays for secretion at CCVs could generate smooth DEJPs like those observed experimentally, a condition was examined in which all the cells in the syncytium received a quantal secretion following a nerve impulse. Although this is an unrealistic proposition for the guinea-pig vas deferens, it was interesting to observe that it did give rise to EJPs in the muscle cells that had a rise time of about 100 ms like those observed experimentally in figure 1*a*,*b* (figure 7). Furthermore, the DEJPs were smooth in all the cells

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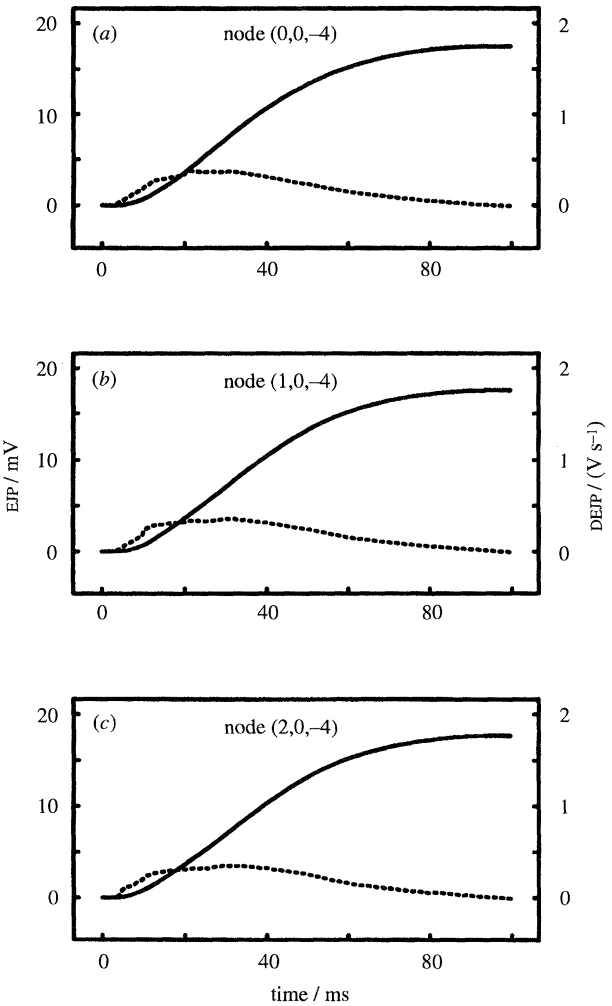

Figure 7. The effect of an exponential distribution of conduction velocities for the different axons giving rise to close-contact varicosities in the syncytium on the time course of the EJP and the DEJP recorded in different cells when every cell in the syncytium receives an innervation. The continuous line gives the EJP and the broken line the DEJP. The results in (*a*), (*b*) and (*c*) are for cells at the positions (0, 0, −4), (1, 0, −4), (2, 0, −4), respectively. The values of τ_g and g_0 were 14.3 ms and 20 nS respectively. The exponential distribution of delays due to the different conduction velocities had a mean of 30 ms and a standard deviation of 30 ms.

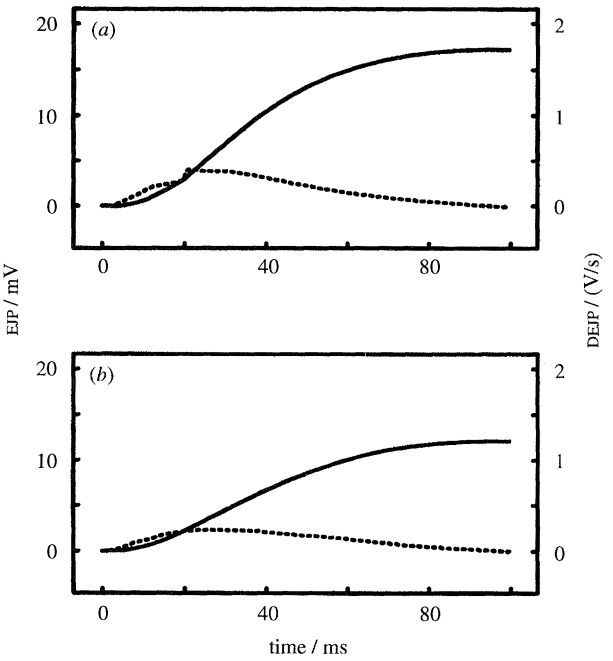

Figure 8. Comparison of the effects of transmitter secretion from close-contact varicosities with that from loose-contact varicosities in the syncytium on the timecourse of the EJP and the DEJP. (*a*) Gives the results for close-contact varicosities on all the cells, in which the peak conductances are drawn from a gamma distribution (mean of 1 nS) and the delays due to different conduction velocities are drawn from an exponential distribution with mean 30 and sd 30; the τ_g for the conductances has value 14.3 ms. (*b*) Gives the results for loose contact varicosities on all the cells; each cell possessed 10 varicosities of which 5 had peak conductances drawn from a gamma distribution with mean 0.1 nS and 5 had peak conductances drawn from a gamma distribution with mean 0.025 nS; the delays and the value of τ_g were the same as in (*a*). The cell recorded from was at position (0, 0, −4).

(figure 7). Comparison of figure 5*c*, in which there was no conduction velocity dispersion (equivalent to intramural nerve stimulation near the recording site), with figure 7 in which there was dispersion according to the exponential distribution of delays (equivalent to hypogastric nerve stimulation) shows a difference in time to peak of about 40 ms. This may be compared with the experimentally observed value of about 30 ms (figure 8 in Blakeley & Cunnane 1979), and lends support to the choice of parameters used in the exponential distribution of delays. However, the problem with these results is that no fast DEJPs are generated, as all the CCVs participate in the slow DEJP.

(*c*) *The effect of variance in the size of quanta secreted by CCVs*

In the above it was assumed that all the quanta gave rise to the same conductance change and this may explain the failure to observe fast DEJPs. This was checked by introducing a gamma distribution of conductances for the effects of quantal secretion from different varicosities in the syncytium. Gamma distributions were used as these give a reasonable description of the distributions of quantal sizes arising from secretion at single varicosities in the mouse vas deferens at normal calcium concentrations of about 2 mM (Macleod *et al.* 1994). This distribution had a mean of 1 nS. Together with the usual distribution of time delays, this gave rise to the EJP shown in figure 8*a*: this rises to a maximum in 100 ms, as observed experimentally, but the DEJP is still relatively smooth, showing little sign of a fast DEJP component.

(*d*) *The effect of probabilistic quantal secretion from CCVs*

Finally, but still in the context of CCVs, a more realistic approach was considered in which all the cells received innervation from a CCV but the probability of quantal secretion from the varicosities was drawn from a beta distribution with mean 0.02 and standard deviation 0.02. These values were chosen as both Brock & Cunnane (1988) and Cunnane & Stjärne (1989) have estimated the probabilities to range between

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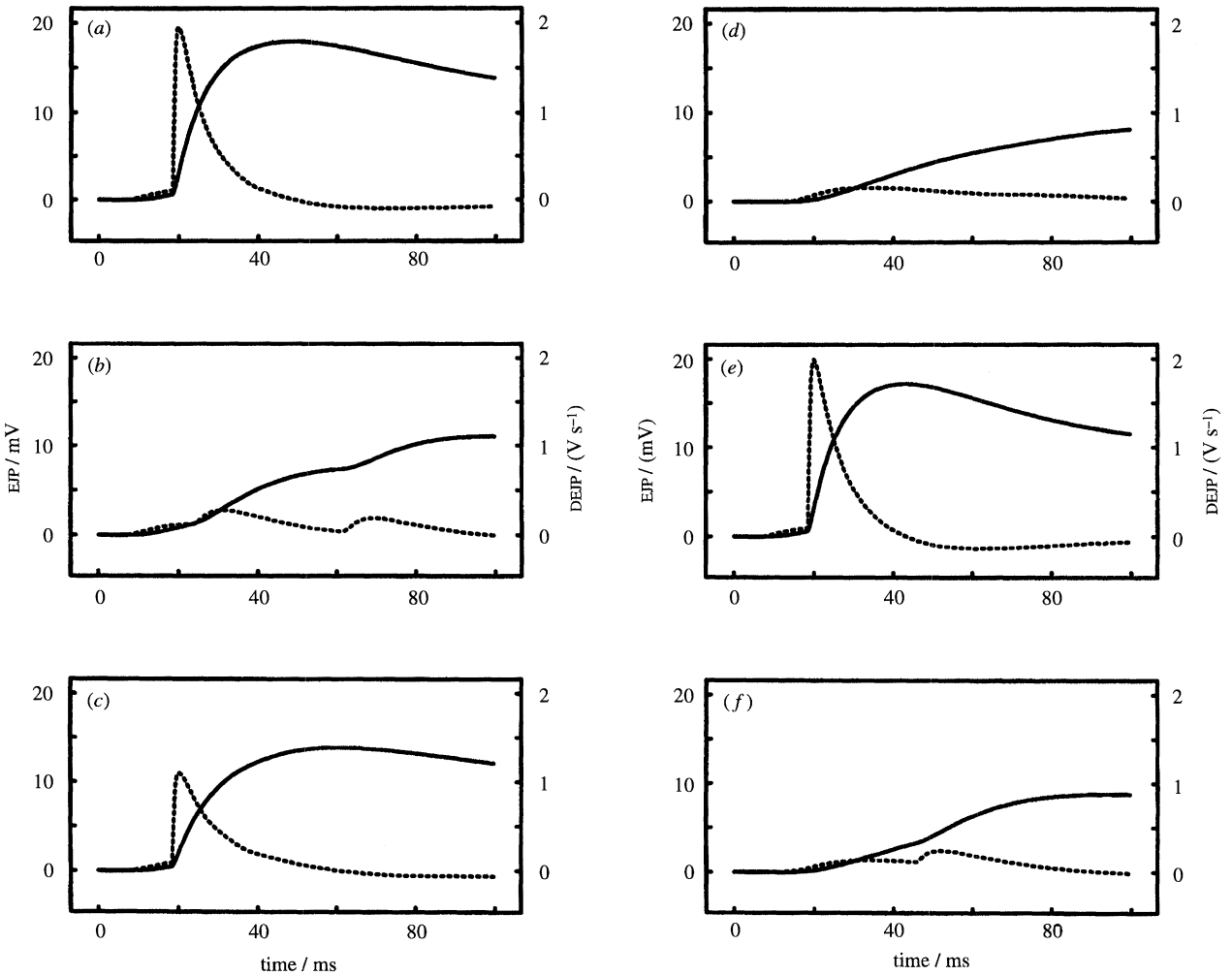

Figure 9. The effect of the probabilistic secretion from close-contact varicosities on the EJP and the DEJP. Every cell in the syncytium receives an innervation from a varicosity with a probability for secretion drawn from a beta distribution with parameters (1, 50). If a secretion occurs it produces a maximum conductance change drawn from a gamma distribution with mean 30 nS and occurring at a latency drawn from an exponential distribution with mean 30 ms. The results are shown for a cell located at $(6, 3, -3)$ following six different impulses (*a*)–(*f*). τ_g was 14.3 ms for all varicosities.

0.002 and 0.03 in the guinea-pig vas deferens; these estimates were based on the frequency of DEJPs at a site during stimulation at 0.5 Hz to 1.0 Hz in a calcium concentration of 1.8 mM to 2.6 mM. The beta distribution was used as this has been found to give an appropriate description of the different probabilities for secretion observed over the release sites of nerve terminals (Bennett & Lavidis 1979; Zefirov 1985). This beta distribution of probabilities, together with the distribution of delays and of the conductances associated with the quanta, gave the EJPs for a single muscle cell shown in figure 9, where the six different cases correspond to six different impulses giving rise to different release patterns and quantal magnitudes. Some of the EJPs reached their peak value in about 100 ms (figure 9*b*,*d*,*f*) whereas others peaked in about 60 ms (figure 9*a*,*c*,*e*), as is observed experimentally. However, even under these circumstances, the slow DEJPs were generally bumpy (figure 9*b*,*f*), reflecting the inputs from nearby cells that received direct quantal inputs. More significantly, in the case where the cell recorded from did not receive a quantal input (figure 9*b*,*d*,*f*) the peak membrane potential was significantly lower (9 mV or less compared with 15 mV or more), whereas in the experimental results (figure 1) about the same peak membrane potential is reached regardless of whether a fast DEJP component is present or not. Thus none of the different approaches to the temporal and spatial distribution of quantal secretion from CCVs has been able to give the form of the rising phase of the EJP as indicated by the shape of the DEJP.

5. THE CONTRIBUTION OF LCVS TO THE RISE TIME OF THE EJP

(*a*) *The timecourse due to slow transmitter diffusion*

Although it has been argued that it is unlikely that the diffusion of transmitter from the muscle bundle after its release from all the varicosities in the bundle contributes to the conductance changes that give rise to the EJP in the guinea-pig vas deferens (see §1), it is of interest to see what might be the effects of varicosities far removed from the muscle cells (several micrometres; see Hille 1992). To this end, and ignoring the decline

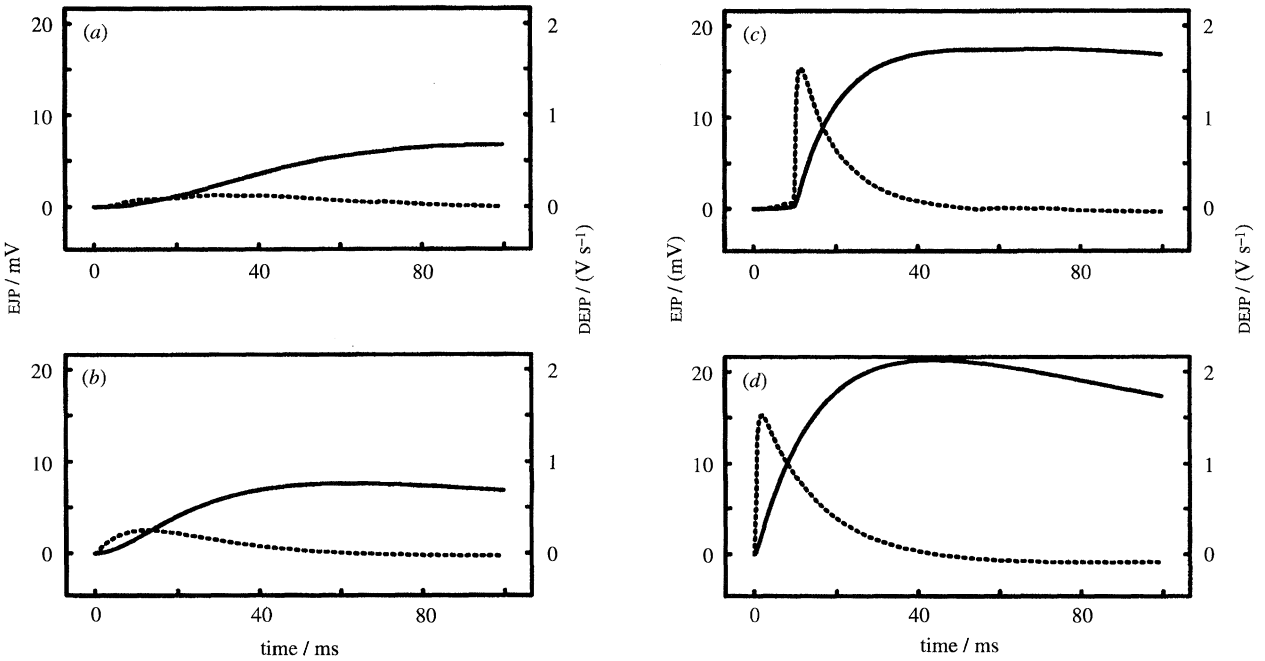

Figure 10. The effect of distant varicosities and of close-contact varicosities on the timecourse of the EJP and of the DEJP. (*a*) Shows the results for a very slow τ_g for the conductance change (τ_g = 33.3 ms) in every cell in the syncytium, meant to simulate the effects of diffusion of transmitter from a large number of distant varicosities; the conduction velocities for the axons subserving these varicosities had an exponential distribution giving rise to a exponential distribution of delays with mean 30 ms and standard deviation of 30 ms and each varicosity produced a conductance change of 0.75 nS. (*b*) The same as in (*a*), except that there was no asynchronous transmitter secretion; that is, all the conduction velocities were the same. (*c*) The effect of superimposing an innervation from close-contact varicosities on every cell in the syncytium with probability of secretion drawn from a beta distribution with mean 0.02, on the slow diffusion of transmitter from distant varicosities onto all the cells in the syncytium as in (*a*); the peak conductance for the close-contact varicosities were drawn from a gamma distribution with mean 30 nS and both the close-contact varicosities and the distant varicosities had the same exponential distribution of delays with mean 30 ms and standard deviation 30 ms. (*d*) The same as in (*c*) except that there was no delay due to different conduction velocities. The position of the cell recorded from in all cases was at (6, 3, −3).

in concentration of transmitter with distance from the source, all the cells in the syncytium were given a very slow input, using an alpha function for the conductance change with τ_g = 33.3 ms, together with an exponential distribution of delays for secretion with a mean of 30 ms. This gives rise to an EJP that peaks in 100 ms, as observed experimentally, and a smooth DEJP (figure 10*a*). Reducing the delays to zero, as occurs approximately when the nerves are stimulated intramurally rather than through the extrinsic nerve supply, reduces the time to peak of the EJP to 60 ms (figure 10*b*), as is observed experimentally in the guinea-pig vas deferens (Blakeley & Cunnane 1979). The question arises as to what happens if CCVs are introduced in addition to the slow diffuse conductance changes. CCVs were placed on every cell with the probability of quantal secretion governed by a beta distribution with mean 0.02 and with the conductances due to the quantal secretions drawn from a gamma distribution with mean 30 nS and with the usual synaptic delays. In this case, the EJPs rose to a peak in 75 ms, and in the case of those cells that possessed a direct input there were inflections on the rising phase (figure 10*c*); the DEJP then showed both a slow time course component as well as a fast component due to the direct input (figure 10*c*). This kind of effect is often observed experimentally (figure 1). Reducing the delays to zero reduced the time to peak of the EJP to 50 ms as before (figure 10*d*). A combination of a slow diffuse input with that of the CCVs can then reproduce all of the experimental observations on the timecourse of the EJP.

(*b*) *The timecourse due to* LCVs

It has already been argued that LCVs provide a way of ensuring that the syncytium reaches equipotential just after the peak of the EJP (3b). Such LCVs can also give the slow component of the DEJP. If, as before, every cell in the syncytium receives an innervation from ten LCVs, then the timecourse of the EJP is such that it rises to a peak in 100 ms and the DEJP is smooth throughout the rising phase of this EJP (figure 8*b*).

6. THE CONTRIBUTION OF COMBINATIONS OF CCVS AND LCVS TO THE RISE TIME OF THE EJP

(*a*) *The effect of variance in quantal secretion from both* CCVs *and* LCVs

If now in addition to the LCVs, CCVs are placed on every 125th cell, and these CCVs give rise to conductance changes that are drawn from a gamma distribution of mean 30 nS, then different cells in the syncytium show EJPs and DEJPs that are very similar to those observed experimentally in the guinea-pig vas deferens (figure 11). In some cells the EJP rises smoothly to a peak and

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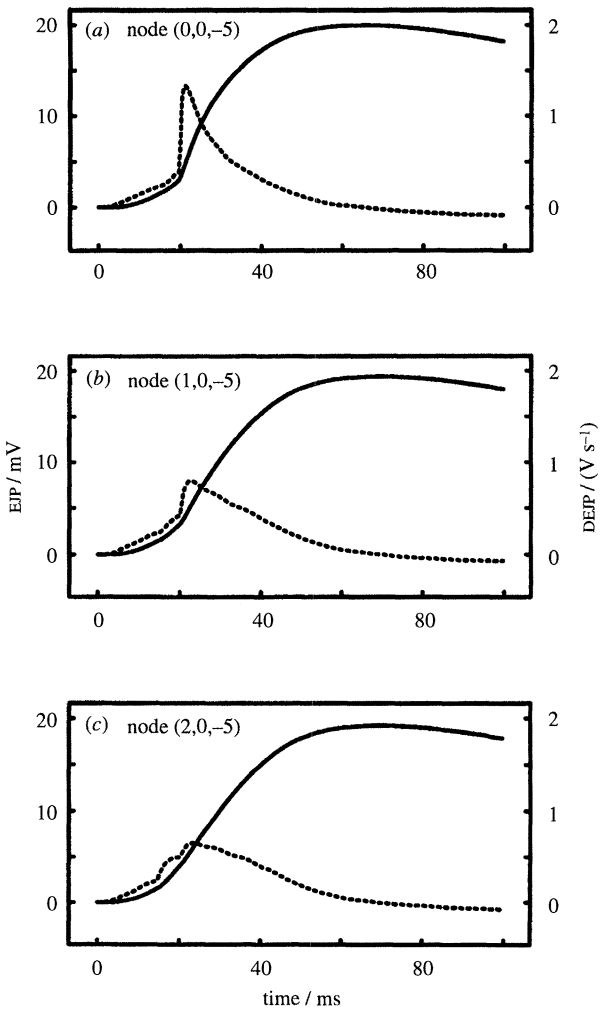

Figure 11. The effect of secretion from both close-contact and loose-contact varicosities on the timecourse of the EJP and the DEJP. One in 125 of the cells received an innervation from close-contact varicosities which had peak conductances drawn from a gamma distribution with mean 300 nS and delays drawn from an exponential distribution with mean 30 ms. All the cells received an innervation from 10 loose-contact varicosities, 5 of which were drawn from a gamma distribution with mean 0.1 nS and the other 5 from a distribution with mean 0.025 nS; the delays were also drawn from an exponential distribution with mean 30 ms. (*a*), (*b*) and (*c*) give the results for different cells, namely at (0, 0, −5), (1, 0, −5) and (2, 0, −5) respectively. $\tau_g = 14.3$ ms for all varicosities.

there is a relatively smooth DEJP (figure 11*c*); in other cells that receive a CCV input the EJP increases with inflexions on its rising phase due to the CCV and the DEJP shows this input as well as the slow input due to the LCVs (figure 11*a*). Other cells show a mixture of these responses (figure 11*b*).

(*b*) *The effect of probabilistic quantal secretion from CCVs and LCVs*

The effect of the stochastic secretion of quanta from both the CCVs as well as from the LCVs on the timecourse of the EJP and the DEJP was also investigated. To this end, the probability of quantal secretion from the CCVs, distributed one per cell, was drawn from a beta distribution of mean 0.02 as before, whereas that for the loose-contact varicosities was drawn from a beta distribution of mean 0.4. The results for a single cell subjected to four different impulses were very similar to those observed experimentally: on some occasions, when the CCV did not secrete, the EJP rose smoothly to a peak in 100 ms (figure 12*a*,*d*); on other occasions, when the CCV did secrete a quantum, the EJP showed inflexions on its rising phase and the DEJP indicated the secretion from the CCV as well as from the LCVs (figure 12*b*,*c*).

(*c*) *The effect of changes in probabilistic secretion from CCVs and LCVs*

The effect of increasing the probability of quantal secretion, as occurs for instance when the calcium concentration is increased, was also determined. All the other parameters were left the same, but the probability for secretion from the LCVs was increased to 1.0 and from the CCVs to a beta distribution with mean 0.1. These are about the changes expected if the calcium concentration was increased from 1.8 mM to 3.0 mM, given a fourth power relationship between the probability of secretion and the calcium concentration (Macleod *et al.* 1993). These increases in probability gave a substantial increase in the EJP amplitude. A series of three impulses to one cell shows the relatively smooth DEJP primarily due to the LCVs with the occasional bumps and fast components due to the direct input CCVs as well as to the radiation into the cell of the electrical effects of CCVs on adjacent cells (figure 13).

7. DISCUSSION

(*a*) *The role of LCVs in reaching equipotential shortly after the peak of the EJP*

Estimates of the time constant for decline of the EJP in the guinea-pig vas deferens vary considerably, between 160 and 450 ms (Bennett 1972; Brock & Cunnane 1992). However, when the EJP time constant is measured in the same vas deferens as that used to estimate the time constant of the membrane, the two values are very similar (Bywater & Taylor 1980). Similar agreement has been obtained for the EJP and the membrane time constant of submucosal arterioles for which the membrane time constant is about 375 ms (Hirst & Neild 1978). In the present work, it is shown that LCVs rather than CCVs are likely to determine that the syncytium reaches equipotential by the time the EJP has declined to about 80% of its peak value. This arises from two considerations. First, each muscle cell would have to receive a very large number of CCVs, given the relatively low probability for secretion (in the guinea-pig vas deferens less than 0.03 in a calcium of 1.8 mM to 2.6 mM, see Cunnane & Stjärne (1989) as well as Brock & Cunnane 1988; for the mouse vas deferens about 0.1 in a calcium of 4 mM, see Lavidis & Bennett 1992, 1993). Such a large number is not at this time substantiated by electronmicroscopy, although it might be when a serial section analysis of individual varicosities is carried out; the syncytium will not reach equipotential by the time the EJP has declined to about

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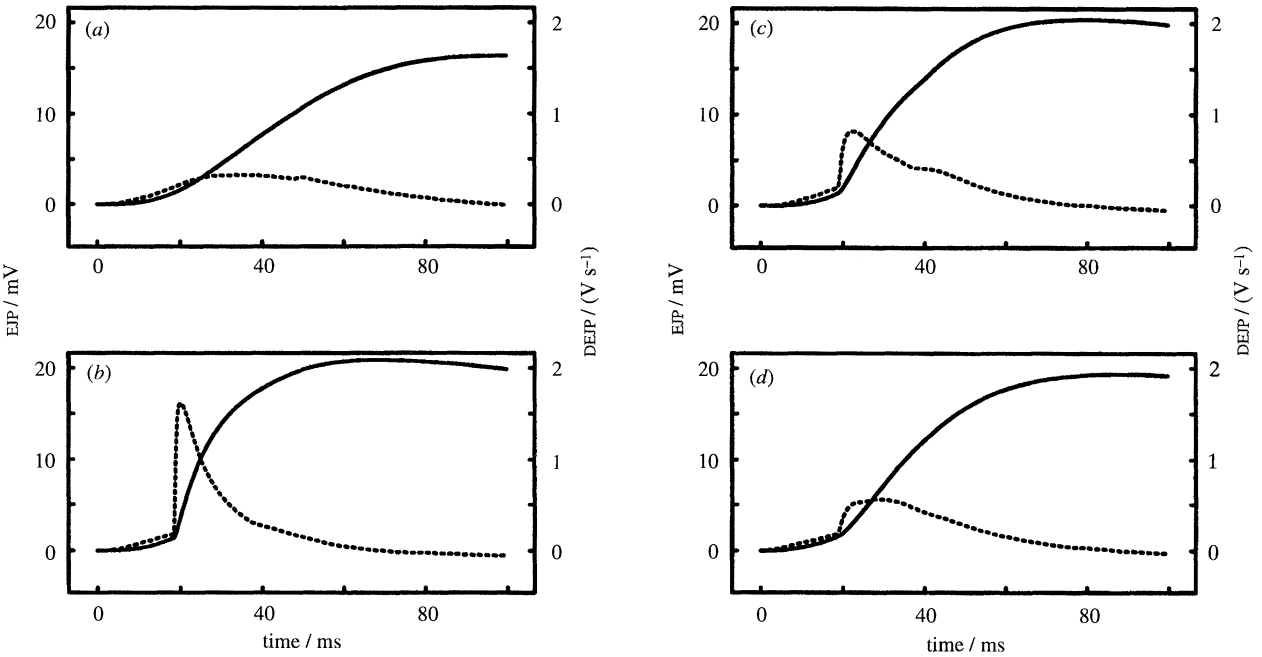

Figure 12. The effect of the probabilistic secretion of transmitter from both close-contact and loose-contact varicosities on the EJP and the DEJP. Every cell in the syncytium receives an innervation from a close-contact varicosity with a probability for secretion drawn from a beta distribution with parameters (1, 50), giving rise to a conductance value drawn from a gamma distribution with mean 30 nS after a delay drawn from an exponential distribution with mean of 30 ms. Each cell in the syncytium also receives an innervation from twenty loose-contact varicosities, each of which secretes with a probability drawn from a beta distribution with parameters (2, 3) after a delay drawn from an exponential distribution with a mean of 30 ms; ten of the loose-contact varicosities produce a conductance change drawn from a gamma distribution with mean 0.1 nS and the other ten from a gamma distribution with parameters (120, 3). (*a*), (*b*), (*c*), and (*d*) give the results for four different impulses to cell $(6, 3, -3)$. $\tau_g = 14.3$ ms for all varicosities.

80 % of its peak value without about one CCV secreting per muscle cell. Second, failure to produce such a high density innervation leads to large differences in the peak size of the EJP in different cells within the syncytium, and this is not observed (Bennett 1972; Bywater & Taylor 1980).

It has been suggested that since extracellular potential measurements fail to detect any current flow shortly after the peak of the EJP is reached, then the declining phase of the EJP must be passive, and therefore does not involve a slowly declining component due to the diffusion of transmitter through the muscle bundle (Cunnane & Manchanda 1988, 1989). However, there can still be a slow diffusional component of transmitter throughout the muscle bundle that would give rise to a substantial elongation of the EJP without being detected with extracellular electrodes. The present work shows that if such a slow transmitter diffusion acts on all the cells in the syncytium then they are at equipotential, and so there is no extracellular current flow that can be detected. This argument is, however, dependent on the appropriateness of using a lumped resistance–capacitance representation for individual cells in the syncytium, each of which is coupled to its six nearest neighbours, except at the muscle surface (Bennett *et al.* 1993). If a distributed equivalent circuit should prove to be more appropriate, and if the coupling between smooth muscle cells in the guinea-pig vas deferens is more sparse, then the above conclusion may have to be revised.

(*b*) *The role of LCVs in generating the slow component of the EJP rising phase*

It is not possible to obtain fast and slow components in the DEJP if the cells in the syncytium receive only CCVs and all these secrete about the same amount of ATP in a quantum onto the same density of receptors. If all of these varicosities secrete a quantum, and these secretions occur with different time delays as a consequence of different conduction velocities, then the slow component of the DEJP can be generated but not the fast component; this problem arises independently of whether or not the quantal secretions give rise to a gamma distribution of conductances. If, on the other hand, the probability of quantal secretion from these varicosities is relatively low then fast components of varying amplitude can be observed, but these are spread out over the entire distribution of delays so that the smooth slow component of the DEJP, which is always observed experimentally, does not occur. An alternative approach for obtaining the slow component of the DEJP is to give each cell in the syncytium a large number of relatively small conductance changes compared with those produced by quantal secretion from a CCV. Such conductance changes might occur as a consequence of quantal secretion from varicosities that surround muscle cells but do not form close-contacts; these have been termed loose-contact varicosities. However, such low conductance changes may occur by other means. For example, the density of receptors beneath CCVs may vary considerably, with

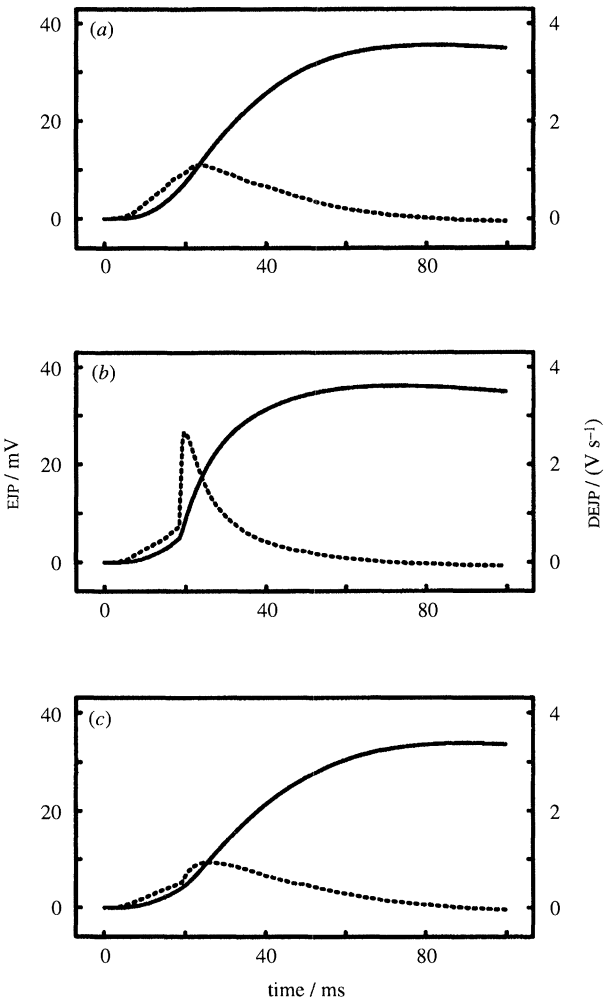

Figure 13. The effect of increasing the probabilistic secretion of transmitter from both close-contact varicosities and loose-contact varicosities, over that given in figure 12, on the EJP and on the DEJP. The probability for secretion from the close-contact varicosities was raised to a beta distribution with parameters (1, 10) and the probability for secretion from the loose-contact varicosities was raised to 1.0. All other parameters were the same as those in figure 12. $\tau_g = 14.3$ ms for all varicosities. (*a*)–(*e*) give the results for three different impulses to cell (6, 3, −3).

only a few CCVs having a high density and therefore a high conductance with the rest consequently having a low conductance. It has recently been suggested that such variations in the density of receptors occur between boutons at synapses on hippocampal granule cells (Edwards *et al.* 1990). However, the distribution and density of P_{2x} receptors at CCVs is not known (Bo & Burnstock, 1992). Another possibility is that the quantal size is not given by a gamma distribution, as the amount of ATP released from varicosities may fall into two different size classes. Both small and large vesicles exist in the sympathetic varicosities of the vas deferens, with the former about twenty times more numerous than the latter in the rat vas deferens (Klein & Lagercrantz 1981). If the small and light particulate fraction of sympathetic nerve terminals corresponds to the small and large vesicle population, then the ratio of ATP to noradrenaline is about the same for both classes of vesicles so that the large vesicles contain about sixteen times more ATP than the small vesicles (Klein & Lagercrantz 1981). If each smooth muscle cell in the guinea-pig vas deferens should prove to have a large number (≈ 40) of CCVs, then it is possible that the large conductance change giving rise to the fast component of the DEJP arises from quantal secretion of the contents of a large vesicle; the slow component of the DEJP would then arise due to quantal secretion from several of the relatively large number of small vesicles. Against this possibility is the observation that the large peptide-containing vesicles seem to require high-frequency stimulation to secrete their contents (Korsakov *et al.* 1988; Whim & Lloyd 1989) and therefore are unlikely to participate in quantal secretion by single impulses during the low-frequency trains (1 Hz) considered in this work.

Finally, we have attributed the small conductance changes to LCVs for the sake of a parsimonious description. However, the distribution of LCVs will not be known until a complete serial-section reconstruction of varicosities at an appropriate degree of resolution is carried out for both the guinea-pig and mouse vas deferens. Furthermore, it should be emphasized that it is surface smooth muscle cells in the guinea-pig vas deferens that have given the electrophysiological results analysed in the present work (figure 1). The distribution of CCVs and LCVs for these cells will therefore have to be determined, not just those deep in the tissue.

Reduction of the conduction velocity delays from an exponential distribution to zero decreases the time to peak of the EJP from about 100 ms to 60 ms. This is observed experimentally if the vas deferens is stimulated via the intramural nerves rather than the extrinsic hypogastric nerve with its comparatively long distances for conduction (Blakeley & Cunnane 1979). The slow component of the DEJP can also be generated by giving each of the cells in the syncytium a very slow alpha function, but we have no biological justification for this approach.

(*c*) *The role of CCVs in generating the fast component of the EJP rising phase*

A combination of LCVs and CCVs can be used to generate all the phenomena associated with both the slow component and the fast component of the DEJP. All the varicosities were given a probability for quantal secretion that was drawn from a beta distribution, with mean 0.02, as this is a typical value for the probability for secretion at individual varicosities (§4*d*). The peak conductance values occurring as a consequence of the secretion of a quantum were drawn from a gamma distribution as this gives a good description of the quantal sizes at a single varicosity (§4*c*). Finally, the dispersion of conductance velocities in different axons was allowed for using an exponential distribution of delays in secretion from varicosities (§4*b*). The combination of these properties for quantal secretion from the varicosities constitutes the final model.

(*d*) *Physiological evidence for the effectiveness of LCVs and CCVs*

The question arises as to the physiological evidence for the effectiveness of LCVs. Observations on the action

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of noradrenaline on the toad heart are difficult to reconcile with the idea that noradrenaline secreted from a varicosity is able to diffuse some distance to act on receptors well removed from those in its immediate vicinity. In this preparation the action of applied noradrenaline to the pacemaker cells is different to that of sympathetic nerve stimulation: in the former case both the amplitude of the pacemaker action potentials as well as the rate of diastolic depolarization is increased whereas in the latter only the diastolic depolarization is changed (Bramich *et al.* 1990). If adrenaline is the transmitter then it appears that on secretion from varicosities it acts on a different pool of receptors to that of the exogenously applied noradrenaline. This suggests that the neurally secreted transmitter only acts locally and does not diffuse to extrajunctional receptors, although the distance over which the local action is exerted may be several hundred nanometres. Similar evidence for junctional and extrajunctional muscarinic receptors has now been obtained for the action of acetylcholine in the heart: stimulation of the vagus nerve to the toad heart reduces a sodium current that makes a major contribution to the depolarization that accompanies diastole; in contrast, exogenous acetylcholine increases a potassium current that leads to hyperpolarization of the muscle cells (Campbell *et al.* 1989; Bywater *et al.* 1989).

It seems likely that the transmitter responsible for the EJP in the vas deferens is ATP and that this has access to a set of purinoceptors different to those on which exogenous ATP mostly acts. For example, suramin blocks the EJP (Sneddon 1992; Karunanithi *et al.* 1993) as well as the contractile effects of the P_{2x} receptor agonist α, β-methylene ATP (Dunn & Blakeley 1988), whereas it only blocks the effects of low concentrations of exogenously applied ATP (von Kugelgen *et al.* 1990). This suggests that exogenous ATP activates a suramin-insensitive site in addition to the P_{2x}-receptors at the site of secretion of ATP. Experiments using the pressure ejection of ATP onto muscle cells of the vas deferens support this idea of different purinoceptors for the endogenous secretion of ATP compared with that for exogenous application: thus the minimum latency for the activation of current on pressure ejection of ATP is about 38 ms compared with that of a few ms for the activation following secretion from the varicosities (Cunnane & Manchanda 1988; Åstrand *et al.* 1988). Furthermore, the channels opened by exogenous ATP have small conductances (Benham & Tsien 1987; Benham 1992) whereas estimates of the number of ATP molecules secreted in a quantum are so small as to suggest that they must act on high conductance ATP channels to give rise to the fast (10 ms) peaking SEJP (Hirst *et al.* 1992).

(*e*) *The role of* LCVS *in the central nervous system*

The concept of synaptic transmission from varicosities that form loose-contacts in addition to those that form close-contacts has been developed in relation to the secretion of transmitter in the central nervous system. The first ultrastructural analysis of noradrenergic varicosities in the cortex lead to the conclusion that only about 5% of these exhibited typical junctional complexes (Descarries *et al.* 1977). Although this very low figure has been subsequently challenged, there is now evidence from the ultrastructural analysis of serial sections for loose-contact serotonergic varicosities in the spinal cord and the mesencephalic central grey substance (Light *et al.* 1983; Clements *et al.* 1985). Loose-contact cholinergic varicosities have also been identified in the hippocampus (Frotscher & Léránth 1985), as have loose-contact varicosities that secrete oxytocin and vasopressin in the median eminence (for a review of the extent of LCVS and CCVS in the central nervous system (see Buma 1988)). It has recently been pointed out by Hille (1992) that, according to the diffusion laws, a transmitter molecule that lasts for about 200 ms before being metabolized or otherwise inactivated will diffuse for a distance of about 60 μm. Such times seem to hold for certain peptides, such as luteinizing hormone-releasing hormone, which can have a transmitter action after diffusing for about 100 μm following secretion from a varicosity (Yan & Yan 1982).

(*f*) *Junctional and extrajunctional receptors on smooth muscle cells*

A main problem to be answered concerns the distance over which a varicosity can be displaced from a muscle cell and still exert an effect on a local pool of 'junctional receptors'. This may be several hundred nanometres, so that both LCVS as well as CCVS secrete onto junctional receptors. The question arises then as to the distribution of the junctional and extrajunctional purinoceptors in the vas deferens. Although autoradiography of the distribution of α, β-methylene ATP binding sites in the rat vas deferens has now been achieved, this is only as yet at the light-microscope level (Bo & Burnstock 1992). It is not clear then whether CCVS, with their closest distance of approach to the smooth muscle cells of about 50 nm, are the only varicosities to possess junctional receptors, or whether varicosities at greater distances can also possess junctional receptors. A comparatively large number of LCVS, at distances of say up to 200 nm closest approach, could generate sufficient current after acting on high-conductance junctional channels (Hille 1992; Jaeger 1965) to contribute substantially to the EJP. This idea of LCVS is substantiated by the amplitude-frequency distributions of quantal sizes recorded from single visualized varicosities on the surface of the mouse vas deferens: for recordings from some varicosities these follow an approximate gaussian distribution that is separated from the noise level (Lavidis & Bennett 1992); in other cases they follow gamma distributions (Lavidis & Bennett 1993); still others follow skewed distributions with smaller values disappearing into the noise level (Lavidis & Bennett 1992). These observations are consistent with the idea that LCVS contribute a relatively small quantal effect because of their distance from the muscle cells, and therefore give rise to a gamma or skewed amplitude-frequency distribution

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of SEJPs; on the other hand CCVs give rise to a large quantal effect and so possess a more gaussian distribution which is clear of the noise level. It should be stressed, however, that there is as yet no ultrastructural evidence at the required level of serial section resolution for the distribution of CCVs and LCVs in the guinea-pig or mouse vas deferens, or for their distances from muscle cells.

Similar conclusions concerning the relation between CCVs and the amplitude-frequency distribution of SEJPs may be reached in relation to the arterioles of the submucosa (Hirst & Neild 1980). Gamma distributions of these were recorded with intracellular electrodes from short lengths of the arterioles, in which the attenuation of the SEJP amplitudes due to the syncytial properties of the muscle is largely circumvented. These distributions were presumably due to the secretion of quanta throughout the syncytium, and suggest that in this preparation many of the varicosities form close-contacts, for which there is ultrastructural support (Luff & McLachlan 1988). It is clear that autoradiographic evidence is required at the ultrastructural level, using ligands against the different classes of receptors, in order to determine the localization of these receptors in relation to close-contact and loose-contact varicosities. Until this is achieved the contributions of different varicosities to the synaptic potentials will remain undetermined.

8. CONCLUSION

The present work indicates that the EJP in the guinea-pig vas deferens is generated from a few large conductance changes together with a large number of small conductance changes throughout the smooth muscle syncytium. The most parsimonious argument at present is that the large conductance changes are due to quantal secretion from CCVs and the small conductance changes are due to quantal secretion from LCVs. If this is so, then LCVs are likely to play a major role in generating the EJP. There are three arguments in favour of this. First, for the syncytium to reach equipotential by the time the EJP has declined to about 80% of its peak value most cells must receive a quantal secretion and given the low probability for secretion from varicosities (about 0.02) this would seem to require a very large number of CCVs per cell compared with that so far reported, although it is probable that larger numbers will be found when serial sectioning of individual varicosities is carried out. Furthermore, innervation of a large proportion of cells with a CCV would give rise to large differences in the size of the EJP between cells and this is not observed. Second, the usual slow and smooth rise of the EJP cannot be generated from just CCVs if there are only a few of these per muscle cell. Third, the rise of the EJP cannot include intermittent fast components superimposed on a slow component if these are both generated from CCVs. The identification of the necessary conductance changes underlying the EJP with the contributions from CCVs and LCVs awaits determination of the spatial distribution of CCVs and LCVs, their distribution of ATP-containing vesicles, together with their post-junctional receptors in the guinea-pig vas deferens.

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APPENDIX. QUANTAL SECRETION IN A SYNCYTIUM

The following is only an outline of the theory; full details are in Bennett *et al.* (1993).

Let V_{ijk} be the excess membrane potential (above its resting value) for the cell at node ijk. Then V_{ijk} satisfies the differential equation

$$\tau_m \frac{\mathrm{d}V_{ijk}}{\mathrm{d}T} + V_{ijk} = \Lambda_x^2(V_{i+1jk} + V_{i-1jk} - 2V_{ijk}) + \Lambda_y^2(V_{ij+1k} + V_{ij-1k} - 2V_{ijk}) + \Lambda_z^2(V_{ijk+1} + V_{ijk-1} - 2V_{ijk}) - R_\mathrm{m}(V_{ijk} - E_0)g(t)\delta_{(ijk),(pqr)}, \quad (1)$$

where R_m is the membrane resistance, $\mathbf{R}_\mathrm{i}$ and $\mathbf{R}_\mathrm{e}$ are the intracellular and interstitial resistances, respectively, and the space constant $\mathbf{\Lambda}$ is defined by $\Lambda_x = \sqrt{[R_\mathrm{m}/(R_\mathrm{ix} + R_\mathrm{ex})]}$, etc. The membrane time constant is $\tau_\mathrm{m} = R_\mathrm{m} C_\mathrm{m}$, where C_m is the membrane capacitance. Transmitter release occurs at nodes pqr ($\delta_{(ijk),(pqr)} = 1$ if $(i,j,k) \equiv (p,q,r)$ and is zero otherwise) and is governed by a driving potential E_0 and a conductance change given by the alpha function (Jack & Redman 1971; Jack *et al.* 1975; Purves 1976):

$$g(T) = \frac{g_0}{\tau_\mathrm{g}} t \exp(1 - t/\tau_\mathrm{g}), \quad (2)$$

where τ_g is the time constant of decay of the conductance change and g_0 is a constant which can depend on the location pqr. This time constant is related to the membrane time constant by $\tau_\mathrm{g} = \tau_\mathrm{m}/\alpha$, where α is a constant.

This equation is to be solved under the initial condition

$$V_{ijk} = 0, \quad \text{for all} \quad i,j,k \quad \text{when} \quad T = 0 \quad (3)$$

and the boundary conditions

$$V_{ijk} \to 0 \quad \text{as} \quad |i|, |j| \to \infty, \; k \to -\infty, \quad (4)$$

and

$$V_{ij1} = V_{ij0}, \quad \text{for all} \quad i,j. \quad (5)$$

In practice, (1) is solved by using a finite difference scheme for the time derivative and then updating using a 'leapfrog' algorithm; typical mesh sizes are $25 \times 25 \times 12$ nodes. The boundary condition (5) on the surface $z = 0$ is easily applied. The boundary conditions (4) on the remaining surfaces can be imposed in several different ways: no specific conditions ('free' boundary conditions); zero current flow normal to the surfaces ('insulated' boundary conditions); or an equal and opposite current fed back ('mirror-image' bound-

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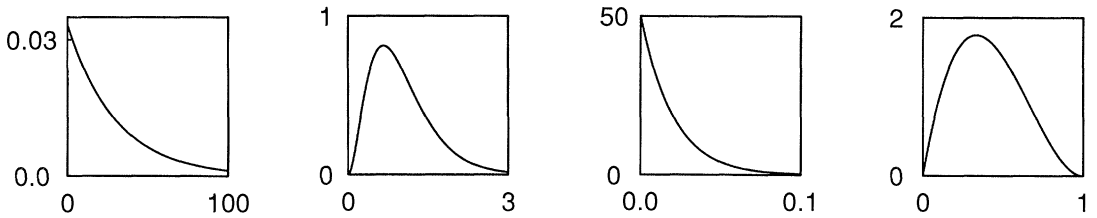
Table 2. *Distributions used in assigning random quantities*

(The probability density (p.d.f.) are illustrated for typical values of the parameters used in the calculations.)

	exponential	gamma	beta	
p.d.f.	$\lambda e^{-\lambda x}$, $x \geqslant 0$	$\frac{\lambda}{\Gamma(r)}(\lambda x)^{r-1}e^{-\lambda x}$, $x \geqslant 0$	$\frac{1}{B(a,\ b)}x^{a-1}(1-x)^{b-1}$, $0 \leqslant x \leqslant 1$	
parameter choice		$r=3$	$a=1,\ b=50$	$a=2,\ b=3$
mean	$1/\lambda$	$3/\lambda$	0.02	0.4
variance	$1/\lambda^2$	$3/\lambda^2$	0.00037	0.04
graph of p.d.f.	$\lambda = 1/30$	$\lambda = 3$		



ary conditions). If only a few varicosities near the origin secrete transmitter, then all these ways are essentially equivalent. If many varicosities secrete transmitter, then in order to approximate this effect in a finite mesh it is necessary to use either insulated or mirror-image boundary conditions: the results from each are almost identical.

The EJP in the muscle cell at node ijk is just V_{ijk}. The DEJP follows by straightforward numerical differentiation with respect to time, using the finite difference formula

$$\frac{dV}{dt} \approx \frac{V(t+\Delta t)-V(t)}{\Delta t}, \tag{6}$$

and a value $\Delta t = 0.4$ ms was found to give adequate accuracy. To calculate the EJC, the extracellular potential V^e_{ijk} is first found from

$$V^e_{ijk} = -\frac{\kappa}{1+\kappa}V_{ijk}, \tag{7}$$

where κ is the anisotropy ratio, defined as the ratio of the interstitial to the intracellular resistance. (This is assumed to be the same in all directions; that is, $\kappa = R_{ex}/R_{ix} = R_{ey}/R_{iy} = R_{ez}/R_{iz}$.) The EJC is the total current flowing out of an electrode placed on the surface of the muscle tissue in the $z = 0$ plane and is given by

$$I_{\text{electrode}} = \Sigma\frac{\Delta V^e}{R_{\text{grid}}}, \tag{8}$$

where ΔV^e is the difference in the interstitial potential between two contiguous nodes in the $z = 0$ plane and the sum is over all grid lines cut by the circumference of the electrode tip.

Various distributions were used in assigning random quantities in the simulations; their main properties, together with diagrams showing a typical shape, are given in table 2.

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