31 Smith, B B , Staib, A , Hynes, J T , *Chem Phys* , 1993, **176**, 521
32 Roy, S and Bagchi, B , *J Phys Chem* , 1994, **98**, 9207
33 Roy, S and Bagchi, B , *J Chem Phys* , 1995, in press
34. Miller, J R , Calcaterra, L T , Closs, G L , *J. Am Chem. Soc.*, 1984 **106**, 3047
35 Mataga, N , Asahi, T , Kanda, Y , Okada, T and Kakitani, T , *Chem Phys* , 1988, **127**, 249
36 Asahi, T. and Mataga, N , *J Phys Chem* , 1991, **95**, 1956.
37. Bixon, M and Jortner, J., *Chem Phys* , 1993, **176**, 467.
38 Tominaga, K , Walker, G. C., Jarzeba, W and Barbara, P F., *J Phys Chem* , 1991, **95**, 10475
39 Nagasawa, Y , Yartsev, A P , Tominaga, K., Johnson, A E. and Yoshihara, K., *J Am. Chem Soc* , 1993, **115**, 7922, Kandori, H , Kemnitz, K and Yoshihara, K , *J. Phys Chem.*, 1992, **96**, 8042
40 Murata, S and Tachiya, M , *J. Am Chem. Soc.*, 1994, **116**, 2434
41 Gayathri, N and Bagchi, B , *J Mol. Struc. (Theor. Chem)*, 1995, in press
42 Gayathri, N. and Bagchi, B , *J. Chem Phys* , submitted
43 Bagchi, B , *Int Rev. Phys. Chem* , 1987, **6**, 1.
44 Kramers, H., *Physica*, 1940, **7**, 284
45 Velsko, S. P., Waldeck, D. H. and Fleming, G R., *J. Chem. Phys* , 1983, **78**, 249
46 Rothenbergere, G , Negus, D. K. and Hochstrasser, R M., *J Chem Phys.*, 1983, **79**, 5360.
47. Flom, S. R , Brearley, A M., Kahlow, M. A., Nagarajan, V and Brabara, S R , *J. Chem. Phys* , 1985, **83**, 1993.
48. Nitzan, A., *Adv. Chem Phys* , 1988, **70**, 489.
49. Grote, R. F. and Hynes, J. T., *J. Chem. Phys* , 1980, **73**, 2715.
50 Bagchi, B. and Oxtoby, D W , *J. Chem. Phys.*, 1983, **78**, 2735.
51. Bagchi, B and Fleming, G R , *J. Phys. Chem.*, 1990, **90**, 9.
52 Sundstrom, V. and Gilbro, T., *J. Chem. Phys* , 1984, **81**, 3463
53. Todd, D C. and Fleming, G R , *J. Chem Phys* , 1993, **98**, 269
54. Nokowa, L., Schwarzer, D , Troe, J and Schreder, J , *J. Chem Phys* , 1992, **97**, 4827
55 Michel-Beyerle, M E., (ed), *Reaction Centres of Photosynthetic Bacteria*, Springer, Berlin, 1991
56. Chandler, D., Gehlen, J. N. and Marchi, M , in *Ultra-fast Reaction Dynamics and Solvent Effects* (eds Gauduel, Y. and Rossky, P J), AIP Conf. Proc , Vol 298, 1993.
57. Dobler, J., Zinth, W , Kaiser, W. and Desterhelt, D , *Chem Phys. Lett* , 1988, **144**, 215.
58 Honig, B , Ebery, T., Callender, R. H , Dinur, U. and Ottolenghi, M , *Proc Natl Acad Sci USA*, 1979, **76**, 2503
59 Austin, R. H , Beeson, K. W., Eisenstein, L , Frauenfelder, H and Gunsalus, C., *Biochemistry*, 1975, **14**, 5355
60. Frauenfelder, H and Wolynes, P. G , *Science*, 1985, **229**, 337.

ACKNOWLEDGEMENTS. The work reported here was carried out in collaboration with my students. I would particularly like to thank Srabani Roy, N Gayathri, Dr A. Chandra and Dr N. Nandi for help and discussions. I thank Prof P. Balaram and Ms N. Gayathri for a critical reading of the manuscript. It is a pleasure to thank Prof Paul Barbara, Prof Graham Fleming, Prof. Mark Maroncelli, Prof A Nitzan, Prof. Iwao Ohmine, Prof Masonari Tachiya and Prof Keitaro Yoshihara for many correspondences and discussions. I also thank Dr K. Bhattacharya, Dr P. K. Das, Dr S. Umapathy, Prof D Mukherjee and Prof V. Krishnan for helpful discussions Prof C. N. R Rao has always been a source of encouragement during the course of the work This work was supported, in part, by the Council of Scientific and Industrial Research, India

Received 26 April 1995; accepted 2 May 1995

Membrane current and potential change during neurotransmission in smooth muscle

Rohit Manchanda

School of Biomedical Engineering, Indian Institute of Technology, Powai, Bombay 400 076, India

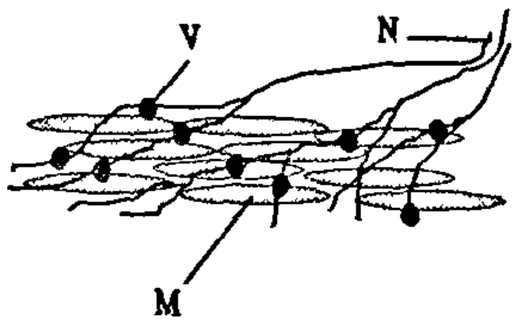
Smooth muscle cells are electrically coupled to one another in a syncytium, and this renders their electrophysiology during neurotransmission strikingly different from that at other synapses. The postjunctional depolarizing responses of sympathetically innervated smooth muscle such as the vas deferens, particularly, the excitatory junction potentials (EJPs), possess intriguing properties which for several years have resisted explanation. A principal issue has been the temporal relationship of transmitter-generated membrane current to the resulting potential change, which seems to differ depending upon whether transmitter release is spontaneous or is nerve-stimulation-evoked. Accordingly, smooth muscle electrical properties appear to change with different patterns of transmitter release. Until some years ago this relationship was an area of uncertainty, firstly because transmitter-activated membrane current could not be measured directly and secondly because intracellular membrane potential measurements gave rise to conflicting results. Many of the uncertainties have now been resolved with refinements in techniques of measurement that have allowed membrane current time course during neurotransmission to be estimated. As a result, our understanding of smooth muscle electrical properties has been clarified and deepened. These developments are outlined in this review, and it is shown how our comprehension of neurotransmission has at every stage been influenced strongly by the techniques adopted for investigation.

THE electrophysiology of neurotransmission from autonomic nerves to smooth muscle has consistently been more challenging to study than somatic neuromuscular transmission. To a large extent this owes to the technical difficulties involved in making electrical recordings from smooth muscles. It is relatively problematic to record intracellularly from individual smooth muscle cells, which are just 2–5 μm in diameter, in contrast to skeletal muscle cells, whose diameters can be 50–150 μm. One requires to use microelectrodes with very small tip diameter ($\leqslant 0.2$ μm) and high tip impedance (50–200 MΩ). Further, when operating at these submicroscopic ranges the recording apparatus is exquisitely sensitive to mechanical vibration. Finally, when recordings are obtained their interpretation in terms of tissue electrical properties often rests on uncertain ground. This is because smooth muscle, as outlined below, is a complex electrical syncytium with cells interconnected to one another in three dimensions[1,2], and their electrical properties are poorly understood. Hence, even the primary depolarizing electrical responses of smooth muscles during neurotransmission, the excitatory junction potentials (EJPs), are not satisfactorily understood. In many cases these responses continue to be explored at the phenomenological rather than at the analytical level. By contrast, the processes involved in transmission at skeletal muscle have been rather thoroughly elaborated[3,4], starting from the end-plate potential down to the gating properties of the transmitter-activated receptor–ion-channel complex and the explanation of the end-plate currents in terms of channel function[5,6]. However, in recent years considerable progress has been made in our understanding of the generation of smooth muscle EJPs as well as the membrane currents underlying them. Some of this owes to the application of novel electrical recording methods to smooth muscle[7,8], and some to theoretical work. Several interesting observations and hypotheses have arisen, and it will be the aim of this article to review these developments. To set them in context, the historical development of ideas on the electrical properties of smooth muscles during neurotransmission will first be outlined. This will include a discussion of the 'cable' properties of smooth muscle, and the factors determining current spread and membrane potential development during EJPs. Since the field is now quite extensive, attention will centre on the properties of sympathetically innervated smooth muscle, particularly the vas deferens, which today provides an interesting and fruitful territory of research. Action potentials in smooth muscles will not be discussed, arising as they do subsequent to junctional transmission. For a survey of this topic the reader is referred to the detailed reviews of Tomita[2] and Huizinga[9].

Syncytial nature of smooth muscle and its innervation

Smooth muscle cells are well known to be electrically interconnected to one another to form what is known as a 'three-dimensional syncytium'[10–14]. Bozler[15] in 1948 was the first to suggest the syncytial nature of smooth muscle. His hypothesis was validated by the demonstration that injected current can flow, and membrane potential changes can be recorded, along smooth muscle tissues over distances which are considerably greater than the average individual cell length. The spread of current and potential occurs presumably through intercellular pathways of low resistance[2,10,16,17]. The syncytial organization of smooth muscle cells renders their electrophysiology in many respects different to that of other kinds of cells, e.g. skeletal muscle fibres, which are electrically isolated from their neighbours.

a Autonomic–smooth muscle



b Somatic–skeletal muscle

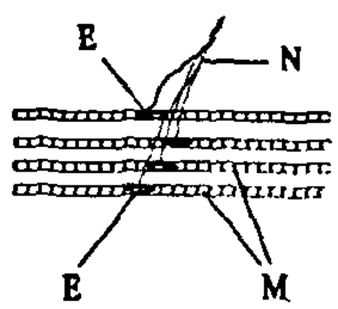


Figure 1. Comparison of patterns of innervation at the autonomic (*a*) and skeletal (*b*) neuromuscular junctions. N: nerve fibre, M: muscle cell; E: end-plate; V: varicosity. Smooth muscle cells are shown relatively shortened in length (e.g. in relation to varicosity diameter) for illustration.

The pattern of innervation of smooth muscle by autonomic nerves also differs from that of skeletal muscle by somatic nerves. As shown in Figure 1 *a*, the innervation of many smooth muscle organs is of the 'distributed' kind, that is, each cell may receive input from varicosities on more than one terminal nerve fibre and any terminal fibre may innervate several cells[14]. The result is a mesh of innervation known as the 'autonomic ground plexus', varicose in appearance, in which the points of transmission are thought to be the periodic axonal swellings, the varicosities. Some cells are innervated closely by the varicosities (contact distance < 20 μm) and others not[14,18]. In contrast to this meshwork, each skeletal muscle fibre receives a well-defined, localized innervation from a somatic motoneurone that forms a discrete 'end-plate' (Figure 1 *b*) where neurotransmission occurs[3–5]. Each skeletal muscle fibre is supplied by the terminal branch of only one somatic motor axon, and usually has just one end-plate.

As a consequence of the electrical interconnections between smooth muscle cells and of their complex innervation, any membrane potential change (such as an EJP) recorded in a particular cell will reflect not simply the response of that cell alone, but also the response, passively propagated to it, of neighbouring cells. Spatial and temporal summation of its own response may occur with the responses of neighbouring cells[11]. The extent of this 'pick-up' will depend largely on the electrical prop-

erties of the interconnecting pathways between the cells. For these reasons it is problematic to analyse electrical events at the level of individual smooth muscle cells in the kind of detail that has characterized the investigation of skeletal muscle, in which the electrical responses of an individual muscle fibre are independent of the responses of neighbouring cells.

Passive electrical properties

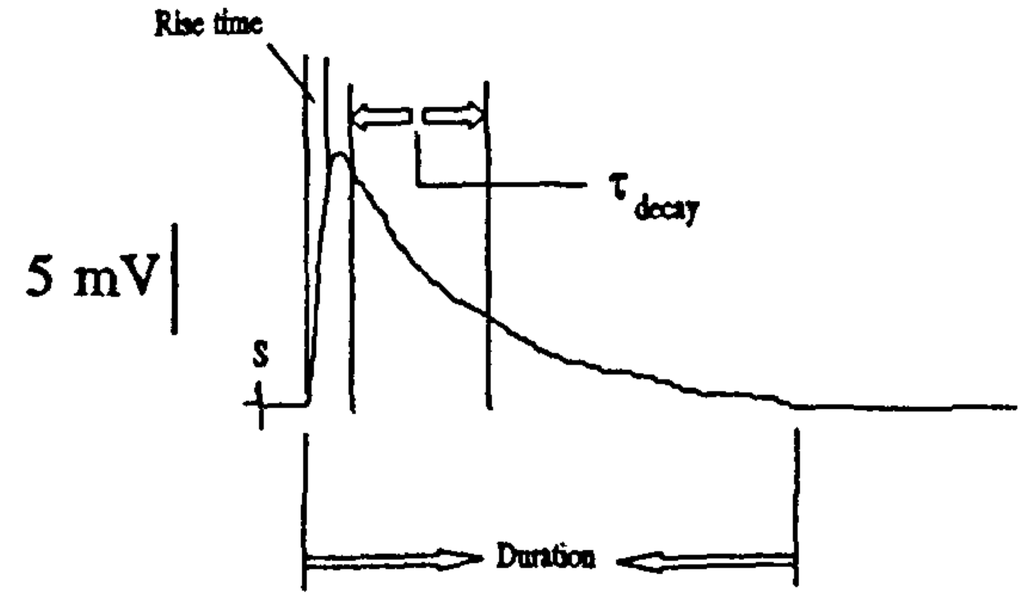
The passive electrical properties of a cell or a tissue, such as its membrane resistance and capacitance, and its length and time constants, are important determinants of the electrical response of the cell to any given input, since they determine its impulse response[19–21]. Thus, the amplitude, time course and spatial spread of a junction potential – be it the EPP in skeletal muscle, the excitatory or inhibitory postsynaptic potentials in neurones, or an EJP in smooth muscle, all of which are produced by neurotransmitter-activated transmembrane current – will depend largely on the passive properties. So will action potential properties such as conduction velocity[22,23].

Junction potentials usually have relatively rapid rising phases and slower, usually exponentially decaying, falling phases (Figure 2 *a*). In many kinds of cells the falling phase of the junction potential decays with a time constant that is equal to the cell's membrane time constant, τ_m. This indicates that the junction potential decay is a purely passive electrical process. The active part of the process, i.e. the neurotransmitter-activated membrane current, is relatively brief. Its duration is often of the order of the rise time of the junction potential (Figure 2 *b*). Transmitter-activated membrane current serves to discharge the membrane capacity and produce the rising phase of the junction potential, with the subsequent decay of the potential following the time course of passive recharging of the membrane capacity, at a rate determined by τ_m (refs. 5, 24). Thus, the time constant of decay, τ_{decay}, of the junction potential equals τ_m (Figure 2).

These properties are in many respects similar to those of potential changes across the capacitor in a parallel resistance–capacitance circuit in response to the flow of current through it. Based on this general principle, a standard method of evaluating the factors governing the time course of a junction potential has been to estimate τ_m and compare it with τ_{decay} of the junction potential. If the two values are similar, then the membrane currents and potential are held to behave as outlined above. If τ_{decay} exceeds τ_m, other factors – such as a duration of transmitter action greater than τ_m – have to be considered[25].

An approach such as this should in principle have revealed readily the elements of junctional processes in smooth muscle. As ever, though, smooth muscle presents bedevilling complications. One is that in a

a. Junction potential



b. Junction current

Figure 2. A typical junction potential (*a*) and its underlying junction current (*b*) as observed at a variety of synapses S stimulation artefact Parameters of analytical interest are indicated Note that membrane potential change is prolonged compared to membrane current, because of the capacitive properties of the cell membrane The downward current trace indicates inward membrane current Typical amplitudes and time courses observed at the skeletal neuromuscular junction are indicated by the calibration bars, however (as will be seen in subsequent figures), values may vary depending upon the cell type under investigation It should also be noted that if junction current time course were measured using extracellular recording, its amplitude calibration would be in volts rather than in amperes

syncytium, τ_m itself varies under different conditions of determination. The estimation of τ_m in simpler structures (those approximating linear one-dimensional cables, such as skeletal muscle fibres and axons) has been relatively straightforward. One injects rectangular pulses of constant current at a point inside the cable and measures the resulting membrane potential changes (the subthreshold or electrotonic responses) at increasing distances from the point of injection[3,5]. τ_m, and other passive parameters such as membrane resistance, capacitance and length constant, λ_m, can then be evaluated using the well-known properties of the cable equation[21].

By contrast, current injection at a point in the smooth muscle syncytium (i.e. intracellularly in a single cell) results in no detectable membrane potential change even in its immediate vicinity[11,26]. The reason for this is thought to be a very rapid spatial dissipation of injected current through the multiple shunts offered by intercellular electrical pathways in three dimensions, so that no appreciable fraction of it flows to any neighbouring area. Thus, smooth muscle does not exhibit cable properties with intracellular current injection. However, when it is subjected to current injection through large

MEASUREMENT OF CABLE PROPERTIES

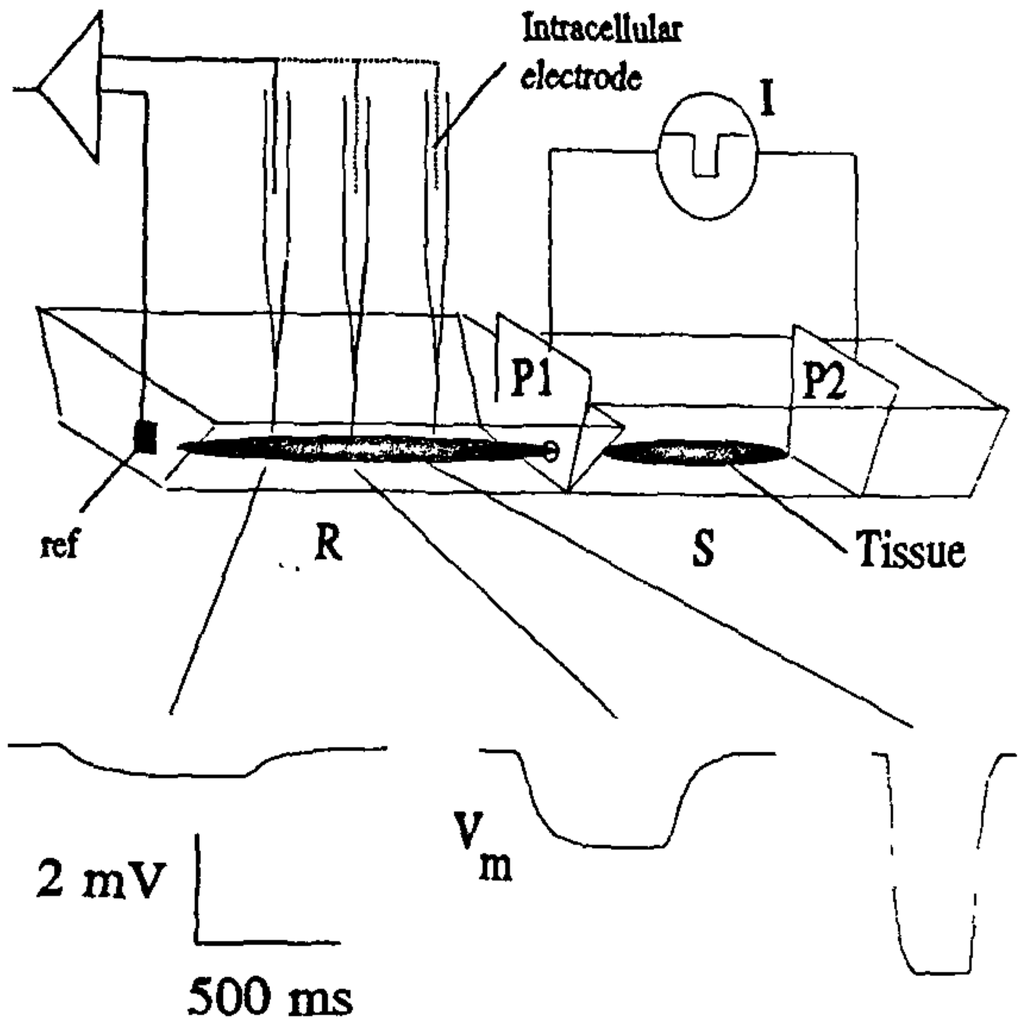


Figure 3. Partition stimulation chamber used for investigation of smooth muscle cable properties R: recording chamber; S: stimulating chamber; P1, P2: metal plates for stimulation and partition; I: current source, V_m: membrane potential change at different distances from plate P1, recorded intracellularly (with reference to indifferent electrode 'ref') The scale is exaggerated V_m responses may be recorded up to only a few mm from P1 Also there would be a tight fit between the tissue and the hole in P1 Amplitude and time scales are appropriate for guinea pig vas deferens Adapted from ref. 16.

extracellular electrodes, smooth muscle does, surprisingly, start to exhibit cable properties, so that membrane potential changes are recorded at a distance from the area of current injection. Particular mention may be made of the 'partition stimulation' method first employed by Tomita[10,11] and Abe and Tomita[16]. The experimental arrangement is shown in Figure 3. The cylindrical smooth muscle organ is drawn through a circular hole of matching diameter in a metallic plate (P1) which forms a plane at right angles to the longitudinal axis of the tissue. The plate also separates the organ bath in which the tissue is placed into two compartments electrically insulated from each other. Current is passed extracellularly between this plate and another plate electrode (P2) in the stimulating compartment (S), while in the recording compartment (R) membrane potential responses are measured intracellularly. As shown in Figure 3, passive (electrotonic) responses can now be detected in tissue cells up to a few millimetres from the plate, and a quantitative evaluation of these responses indicates that the organ now behaves as a one-dimensional cable. An explanation for this change of properties is that when external plate electrodes are used to polarize the smooth muscle cells, the tissue is rendered more or less isopotential in its two radial axes at the stimulation plate. Hence, injected current can only spread in one direction – along the longitudinal aspect of the tissue – and the tissue acquires cable-like properties in this direction[10,11,17]. Some of these properties are: (i) the peak values of the membrane potential responses to external stimulation decay exponentially with distance from the polarizing plate; (ii) the temporal properties of the membrane potential changes at different distances can be described by linear one-dimensional cable equations.

The electrotonic responses thus measured can be used to estimate passive electrical properties such as length constant and time constant of the tissue[11,27]. In the case illustrated (hyperpolarizing current injection) the change of membrane potential (V_m) with respect to both time (t) and distance (x) is given by[16,19]

$$V_m(X,T) = V_m(X=0, T=\infty)\frac{1}{2}\left\{e^{-X}\left[1+\mathrm{erf}\left(\frac{X}{2\sqrt{T}}-\sqrt{T}\right)\right]\right.$$
$$\left.+e^{X}\left[1-\mathrm{erf}\left(\frac{X}{2\sqrt{T}}+\sqrt{T}\right)\right]\right\}, \qquad (1)$$

where $X = x/\lambda_m$ (here $0 < X < \infty$) and $T = t/\tau_m$, x being the distance from the stimulating electrode and t the time from start of stimulation; erf is the error function, defined as

$$\mathrm{erf}(y) = \frac{1}{2\sqrt{\pi}}\int_0^y e^{-\omega^2}\,d\omega. \qquad (2)$$

By comparing the prediction of equation (1) with the observed responses, one can verify the correctness of the values of the length constant and time constant obtained experimentally, and thus estimate the passive membrane properties.

Based on the responses to intracellular and extracellular polarization, various suggestions, often conflicting, have been made about the factors determining the time courses of EJPs, and the relationship of membrane potential change to membrane current, in smooth muscle. These hypotheses are reviewed below.

Smooth muscle junction potentials and their analysis

Two kinds of excitatory potentials are recorded at the synapse between the terminals of the vas deferens nerve postganglionic to the hypogastric nerve and the smooth muscle cells of the vas[28,29]: the evoked and spontaneously occurring junction potentials (the EJP and SEJP, respectively, Figure 4 *a*). EJPs and SEJPs have been recorded in several autonomically innervated smooth muscles, but the most detailed work has been done on junction potentials at the sympathetic neuroeffector junction, particularly in the rodent vas deferens, and in arteries and arterioles.

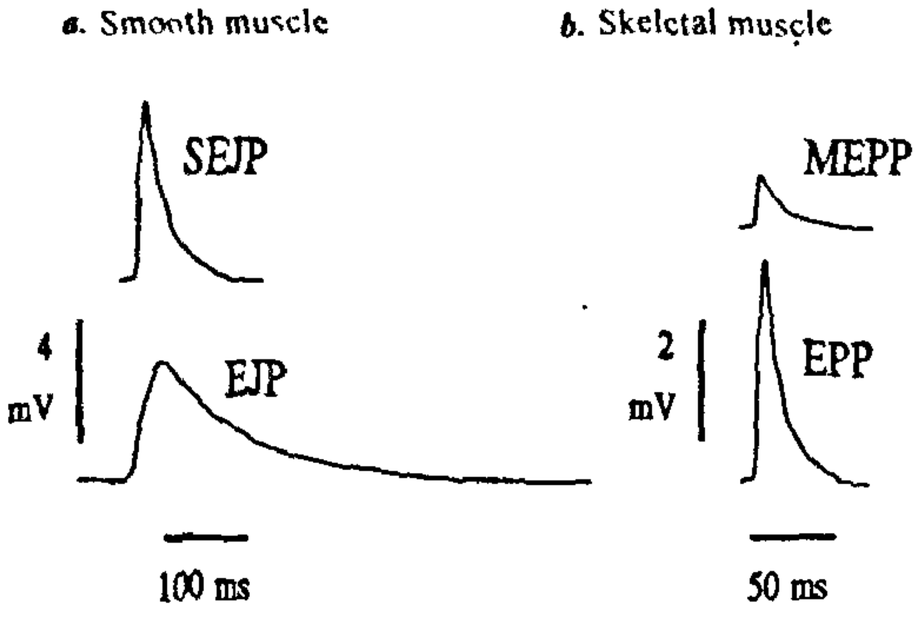


Figure 4. Spontaneous and stimulation-evoked junction potentials seen in smooth (*a*) and skeletal (*b*) muscle Spontaneous potentials: SEJP, MEPP. Evoked EJP, EPP. In smooth muscle the SEJP is briefer than the EJP but can be as large as the EJP. In skeletal muscle the MEPP follows the same time course as the EPP but has a smaller amplitude. Note the different time scales in *a* and *b*

The SEJP occurs in the absence of nerve stimulation and is thought to be caused by the spontaneous release of a quantum of transmitter from a neuronal varicosity. The EJP, on the other hand, is a stimulation-evoked event and is thought to be caused by the release of transmitter throughout the neuronal ground plexus following nerve stimulation[30,31]. Qualitatively, these responses are analogous to the miniature and the stimulation-evoked end-plate potentials (MEPPs and EPPs) observed at the skeletal neuromuscular junction, produced, respectively, by the release of one quantum of transmitter (spontaneously) or of several quanta (following nerve stimulation)[32] (Figure 4 *b*). However, there are important differences, as illustrated in Figure 4. In relation to time courses the most striking one is that whereas the MEPP and the EPP have similar time courses (Figure 4 *b*), the SEJP and the EJP differ by a factor of 5–10 in theirs, the SEJP being briefer[28,33] (Figure 4 *a*). SEJPs usually have durations of 100–150 ms and τ_{decay} of 20–50 ms, whereas EJPs have durations of 0.6–1 s and τ_{decay} ranges from 200 to 400 ms.

Another difference lies in relative amplitudes. Skeletal muscle MEPPs have amplitudes that are normally distributed about the range 0.5–1 mV, and the EPP amplitude is an integral multiple of this quantal level; EPPs are thus always as large as, and normally much larger than, MEPPs[32]. By contrast, in smooth muscle both EJP and SEJP amplitudes are continuously variable from the lowest to the highest levels (0–30 mV), EJP amplitude being graded with stimulation intensity and SEJP amplitudes varying randomly. There is no obvious quantal relationship between the two events, and SEJPs can often be larger than EJPs[28,33] (Figure 4).

Since MEPPs and EPPs have similar time courses, it would appear that the passive electrical properties of skeletal muscle cells remain invariant regardless of whether transmitter release is spontaneous or stimulation-evoked. However, in smooth muscle cells, since the SEJP and EJP follow very different time courses, the passive properties appear to change depending upon the pattern of transmitter release, i.e. whether it is spontaneous and localized to a varicosity or is evoked by nerve stimulation and, therefore, involves several varicosities throughout the ground plexus[11,13].

Using the principle of comparison of τ_m with τ_{decay} of the junction potentials, the decays of the MEPP as well as of the EPP of skeletal muscle were shown to be determined by the passive properties of the skeletal muscle cell membrane. However, in initial studies the decays of the SEJP and EJP in the vas deferens were suggested by various workers to reflect not τ_m but the time course of membrane current, indicating a prolonged duration of action of transmitter at the autonomic neuroeffector junction.

Experimental studies

The SEJP. For the SEJP, evidence for prolonged transmitter action came mostly from studies on membrane potential responses to intracellular stimulation. If the SEJP results from localized transmitter action at a point in a three-dimensional syncytium, then the electrical properties of the smooth muscle membrane during intracellular current injection may be assumed to reflect its properties during an SEJP. A number of workers[11,34–36] have studied the membrane potential responses of smooth muscle cells to intracellular current injection with current-passing microelectrodes. In the guinea pig and mouse vas deferens this response was usually observed to have a time constant of 2–7 ms, which is up to an order of magnitude higher than the time constant of decay of the SEJP (range 20–35 ms). It could even be more rapid than the rising phase of the SEJP, which occupies between 5 and 15 ms.

Thus, the time constant of the membrane in response to local application of current in the syncytium was much too brief to account for the time constant of decay of the SEJP. This excluded the possibility that the passive membrane properties of the vas deferens determined the decay of the SEJP. Hence, for the SEJP it was concluded that transmitter action might continue throughout its duration[10,11].

The EJP. Suggestions about the factors governing EJP time course came primarily from estimation of smooth muscle passive properties using the partition–stimulation method. From the passive responses described by equation (1), Tomita[11] estimated the length constant (λ_m) of the tissue to be about 1.5–2.5 mm. The membrane time constant τ_m of the smooth muscle cells was estimated to be about 100 ms.

The time constant of the declining phase of the EJP (200–400 ms) is significantly greater than this value of τ_m. Hence, it appeared that τ_m was too brief to account for the falling phase of the EJP, rather like the relationship of τ_m during intracellular polarization in relation to the SEJP. It was concluded[11,17,26] that the EJP too might reflect the time course of ongoing transmitter action rather than that of the passive recharging of the membrane capacity.

Thus, until the mid-1970s there seemed to be a consensus that transmitter-activated membrane current during both the SEJP and the EJP might persist throughout the duration of each category of junction potential. This contrasted with the brief time course of membrane current underlying the MEPP and the EPP at the skeletal end-plate. In the last 10–15 years, however, much evidence has been presented which suggests a somewhat different picture for the EJP. Let us consider these developments.

The most pertinent experimental analysis is that of Bywater and Taylor[27], who reinvestigated the passive electrical properties of the guinea pig vas deferens. They showed that the estimation of membrane time constant depended critically on the respective lengths of the tissues placed in the recording and stimulating compartments of the partitioned chamber. In particular, the calculated time constant was shown to be a considerable underestimate of its true value if less than three length constants of the tissue were placed in the stimulating compartment.

Obviating these sources of error, τ_m was estimated[27] with external polarization to be about 270 ms, a value significantly different from the previous estimate (100 ms)[11]. The length constant λ_m (~860 μm) was also shown to be smaller than the earlier estimate[11] of 1500–2600 μm.

Unlike the earlier value, the new estimate of τ_m coincided with the time constant of decay of the EJPs in the guinea pig vas deferens. The decay of the EJP, therefore, appeared to be dictated by the passive membrane properties of the smooth muscle cells. This would indicate a brief duration of transmitter-activated current underlying the EJP. Blakeley and Cunnane[30] and Bywater and Taylor[27] suggested that transmitter action underlying both spontaneous and evoked junction potentials corresponded in duration to the SEJP.

Theoretical studies

Smooth muscle syncytial properties have also been investigated from a theoretical standpoint[10,11,13]. It was shown that if current were injected from a point source into the syncytium (as for the SEJP) then, because of intercellular electrical coupling, the current would dissipate away from the point of injection very rapidly, both spatially as well as temporally[11]. As a result, the time course of the membrane potential change at or near the point of current injection would be similar to that of the current itself. On the other hand, when current of the same time course is injected uniformly throughout the syncytium (as for the EJP), spread of current is restricted and the potential change that develops then is prolonged, lagging considerably behind the current[13].

Purves[13] obtained the time course of the EJP by convolving the impulse response, $h(T)$, of an isopotential circuit with the input, the transmitter-activated current $I(T)$:

$$h(T) = \frac{Q_0}{c_m} e^{-T}, \tag{3}$$

$$I(T) = \alpha^2 T \exp(-\alpha T), \tag{4}$$

where Q_0 is the charge deposited instantaneously on, and c_m the membrane capacity of, unit volume of tissue, T is the normalized time (t/τ_m) and α is a driving function which, as used in equation (4), generates waveforms very similar in shape to synaptic currents observed at a variety of synapses[13,23]. Then the membrane potential response $V(T)$ is given by $V(T) = h(T)*I(T)$, the asterisk indicating the convolution operation. On solving the convolution integral we get the essential part of $V(T)$ as

$$V(T) = \alpha^2 e^{-\alpha T}\left\{\frac{e^{T(\alpha-1)}-1}{(\alpha-1)^2} - \frac{T}{\alpha-1}\right\}. \tag{5}$$

On evaluation, $V(T)$, corresponding here to the EJP, turns out to be considerably prolonged compared to the injected current $I(T)$.

Purves[13] also derived an expression for the voltage change during the SEJP. In this case the impulse response $h(T)$ is given by

$$h(T) = \frac{Q_0 r_i}{8(\pi T)^{3/2}\lambda\tau} e^{[-(R^2/4T)-T]}, \tag{6}$$

r_i being the intracellular resistance per unit volume and R the normalized radial distance.

Equation (6) can be convolved with the expression for transmitter-produced membrane current (equation (3)) and the voltage response derived. It was shown that the resulting voltage change is much briefer than that predicted for the isopotential EJP and, in fact, follows closely the time course of the current itself[13]. Hence, this theoretical work indicated that whereas the EJP is prolonged compared with its underlying membrane current, the SEJP follows the time course of the current.

Recent investigations

The foregoing conclusions can be unambiguously tested only by direct observation of the junction currents that underlie the potentials to establish the relationships between their time courses. One way to record junction

current is to perform a voltage clamp experiment, which has been done on several types of electrically isolated cells, including skeletal muscle fibres and neurones[4,24,37]. However, it is difficult normally to obtain an adequate voltage clamp of syncytial, three-dimensional tissues such as smooth muscle[38]. This is because, as previously described, smooth muscle cells have unsuitable geometries and are electrically interconnected.

Extracellular recording. A second convenient method of estimating membrane current time course is to record extracellularly the potential changes caused by the current sinks created focally at points of transmitter action[39]. Until the late 1980s this method could not be successfully applied to the autonomic-nerve–smooth-muscle junction because of technical problems[7,40,41]. However, in the past few years attempts at extracellular recording have been successful and, therefore, it has been possible to estimate the time course of membrane current underlying the EJPs[7,41–43]. The extracellular electrode used in the initial studies had a large tip diameter (20–50 μm) compared to the intracellular electrode, and was applied to the surface of the vas deferens with slight suction. This allowed the recording of negative-going extracellular potential changes of 20 μV and greater in amplitude. Since these events reflect the time course of membrane current, they were named excitatory junction currents (EJCs)[7].

Simultaneous intracellular and extracellular recordings of the time courses of junction potentials and currents have been obtained[8,33] and have given rise to some conclusive and interesting observations. In the experiments in which the spontaneous events were recorded, cells within a short distance (up to 100 μm) of the rim of the extracellular electrode were probed with the intracellular microelectrode[8,44]. This was necessary because, as mentioned above, the spatial decay of spontaneous potentials is very rapid and the same event could be detected with two electrodes only if they were very close to each other. The experiments unequivocally showed that when the same electrical event was recorded at both the electrodes[44], the time courses of the SEJP and the SEJC (i.e. the potential change and the current flow) were identical[8]. This is shown in Figure 5 for three pairs of SEJPs and their underlying SEJCs, and it is noteworthy that even for very different shapes of the events in Figure 5 *a*, *b* and *c*, potential change and current follow the same time course. The result strongly suggests that the membrane capacity is not charged significantly by the current induced by the action of a single quantum of transmitter, since if it were, membrane potential change would have lagged behind the current.

The nerve-stimulation-evoked EJP and the time course of its underlying EJC were also recorded simultaneously[8,33]. In this case it was observed that the membrane currents underlying the EJPs were brief, having

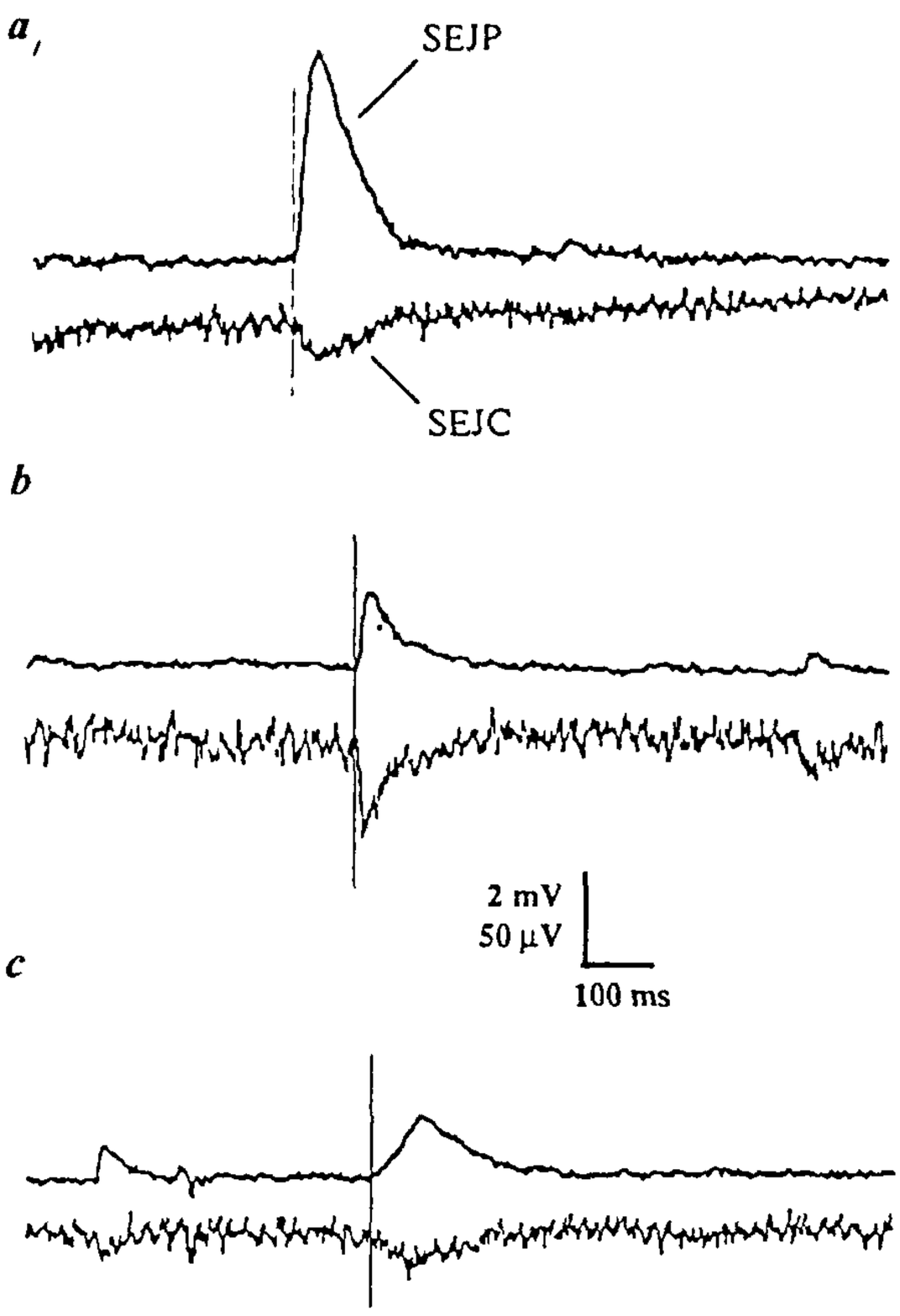


Figure 5. Spontaneous electrical events in smooth muscle (mouse vas deferens) recorded simultaneously with intracellular and extracellular electrodes (upper and lower traces, respectively, in *a*, *b* and *c*) Note the close correspondence between the time courses of membrane potential change (SEJP) and junctional current (SEJC) even for different shapes of the events· the almost linear decays in *a*, the biexponential decays in *b*, and the prolonged rise times in *c*. The reason for the somewhat distorted shapes is probably the mechanical pressure exerted by the extracellular electrode on the tissue[44]. Voltage scale 2 mV for intracellular, 50 μV for extracellular traces

durations (100–150 ms) and time constants of decay (25–40 ms) no greater than of the SEJCs (Figure 6). Hence, the prolonged time constant of decay of the EJPs (250–350 ms) cannot be attributed to prolonged transmitter action, owing, for instance, to slow inactivation or to physical barriers to diffusion[1]. It must instead be attributed to the passive decay of charge from the smooth muscle cells following spatially distributed injection of current into the tissue that changes it from a three-dimensional syncytium into one that is presumably at isopotential[13,33]. If this indeed is the case, the theoretical isopotential voltage response of the tissue (given by equation (5)) should predict successfully the experimental data. Evidence for this is presented in Figure 7. EJPs and EJCs were recorded simultaneously and 80 of each were averaged[33, 44] (Figure 7 *a*). The EJC was

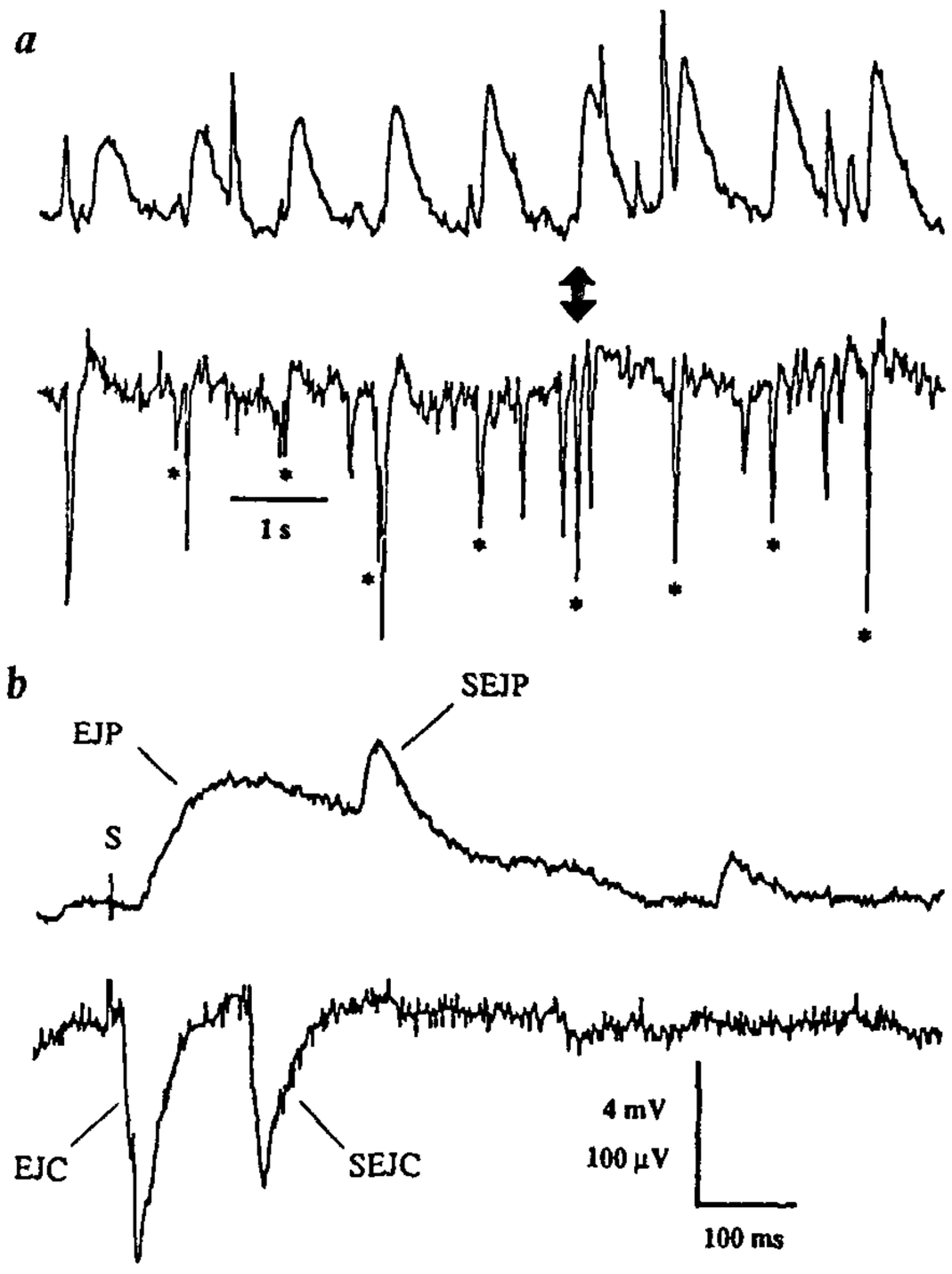


Figure 6. Stimulation-evoked electrical events in smooth muscle (guinea pig vas deferens) recorded simultaneously with intracellular and extracellular electrodes. A series of 9 EJPs is shown in *a*, the underlying EJCs marked with asterisks. In *b*, one of the paired events in *a* (marked with an arrow) is shown on an expanded time scale Note the brief time course of the EJC compared to the EJP. Other deflections in *a* are spontaneously occurring SEJPs and SEJCs; one of each is indicated in *b*. Voltage scale· 4 mV for intracellular, 100 μV for extracellular traces S stimulation artefact

shown to match the form of the theoretical junction current given by equation (3) (Figure 7 *b*). When this current was used as the input to an isopotential system and the voltage response computed, the response agreed very closely with the experimental EJP (Figure 7 *c*). In these recordings, therefore, the tissue appears to be at isopotential during the EJP[13,33]. In further support of this conclusion is the observation that under conditions of field stimulation of the tissue, the estimated membrane time constant of these cells (270–300 ms)[27] is very similar to the time constant of decay of the EJPs.

Bennett *et al.*[45] have carried out a theoretical analysis of the signals that one would expect to obtain with an extracellular surface electrode of the kind used in the studies mentioned above. Their predictions are in very good accord with the EJCs experimentally recorded earlier[8,41,43], and place confidence in the validity of the new measurements.

The quantal relation between the EJP and SEJP

Although the time courses of the SEJP and the EJP are widely different, the events have similar ranges of amplitudes, from just-discernible to threshold levels for action potential generation. The range of amplitudes of SEJPs can be explained by the effect of electrotonic decrement on events generated in a syncytium at varying radial distances from the recording electrode[11,13]. Events generated very close to the recording electrode are recorded as large SEJPs, and those at larger distances as smaller SEJPs. However, the EJP seems not to be composed of an integral multiple of SEJPs, as the quantal hypothesis in its simplest form (derived from the properties of the EPP and MEPP at the skeletal neuromuscular junction[32]) would suggest. This feature of sympathetic neurotransmission, by itself a separate important area of investigation, merits a brief comment. The problem here is that the EJP and the SEJP arise under different prevailing electrical conditions of the tissue (as detailed above). The EJP for most of its time course is a passive electrical event, and only its rising phase is actively determined by the underlying junction current (see Figures 6 and 7). So, a resolution of transmitter release events following nerve stimulation, and electrical identification of sites of release, would require investigation of its rising phase, in contrast to the decay phase, which throws light on the tissue's electrical properties.

Detailed examination of the rising phases of EJPs has revealed that they are not entirely smooth but are marked by inflexions, and that both the slope and the time of occurrence (latency) of these inflexions differ from one EJP to the next[30]. The first time differentials (dV/dt) of the rising phases, therefore, contain sharp peaks corresponding to the inflexions, and are termed 'discrete events' (DEs). DEs were postulated to be indications of the activity of individual transmitter release sites close to the recording electrode, the remainder of the EJP reflecting the background level of depolarization in the surrounding tissue. Rising phases of SEJPs were similarly differentiated to give rise to spontaneous DEs (SDEs).

Using DEs it has been possible to 'fingerprint' different release sites around the intracellular recording electrode by virtue of the differences in amplitudes, time courses and (for evoked events) latencies of the DEs[31]. Evidence from these studies suggests that stimulation-evoked release from an individual electrophysiologically identified release site (possibly a varicosity) is highly intermittent, and monoquantal. That is, in a train of stimuli, only one in several stimuli activate a particular release site. And when a site is activated, the resultant evoked DE corresponds exactly to a particular SDE observed in that cell[31]. It is not an integral multiple of the SDE. Thus, evoked release must be monoquantal if the

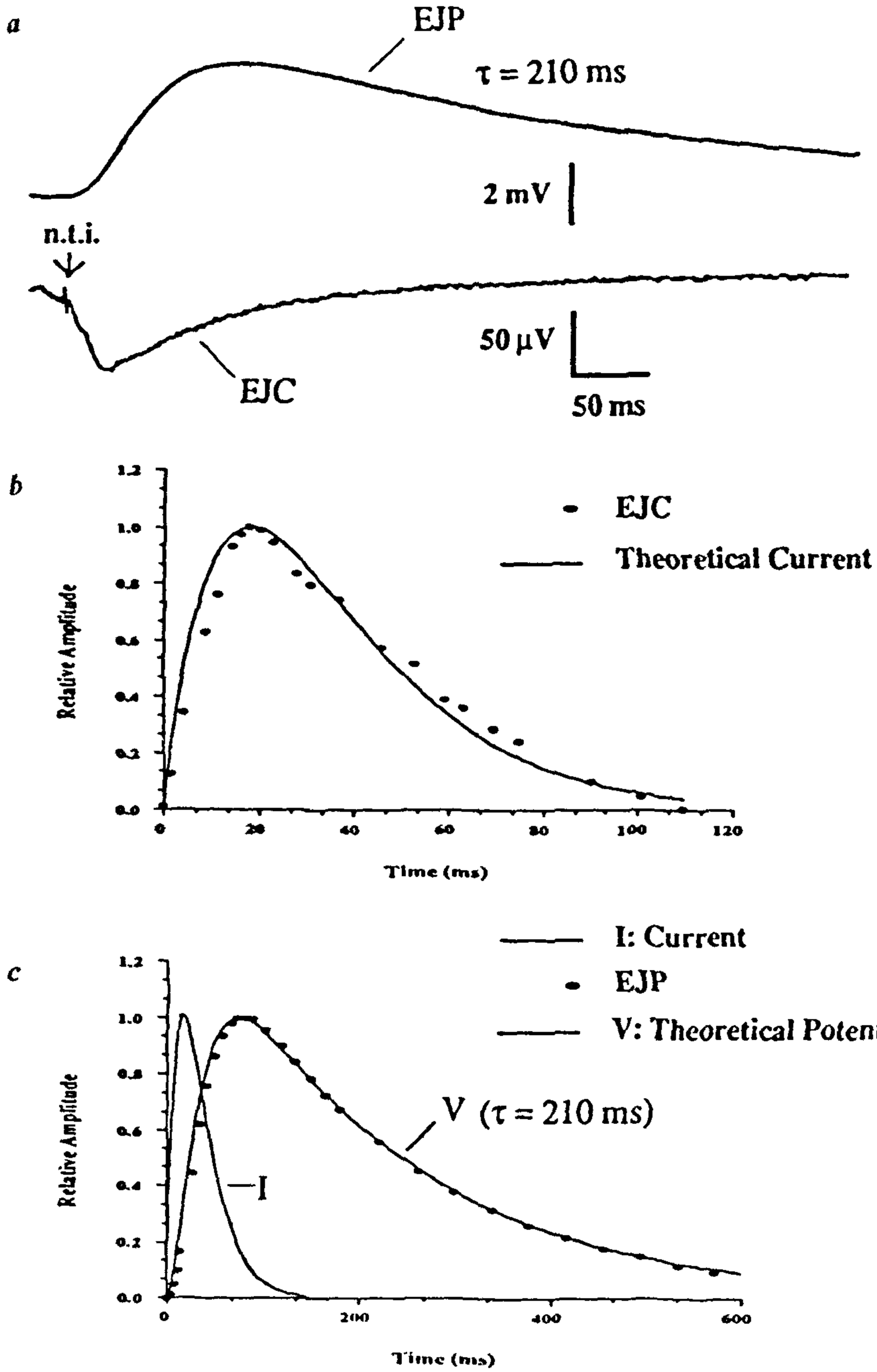


Figure 7. Reconstruction of the EJP from the EJC based on Purves' model[13, 44]: *a*, averages of 80 EJPs and EJCs simultaneously recorded (n.t i. is the nerve terminal impulse recorded extracellularly); *b*, plot of the EJC in *a* (filled circles) compared to theoretical EJC (smooth curve) generated by equation (4) of the text; *c*, plot of experimental EJP in *a* (filled circles) along with theoretical EJP (smooth curve) generated by Purves' model (equation (5) of text) using the underlying EJC (*I*) as the input. Note the agreement between experimental and theoretical time courses. In *b* and *c*, all events have been normalized with respect to their maximum amplitudes in order to facilitate comparison of time courses.

SEJP is held to be a monoquantal event (on which there is general consensus). Analysis of EJCs and SEJCs using the extracellular recording technique has confirmed this hypothesis of intermittent, monoquantal release from release sites[41–43,46]. However, it should be noted that during an EJP several release sites throughout the

ground plexus are activated, and in this sense the EJP is a multiquantal event. But because the activated release sites are spatially dispersed[46], in the EJP's rising phase only the contribution of one of the closest sites is usually recorded intracellularly as a DE, the contribution of more distant sites being attenuated by electrotronic factors. Since release from each of the sites is monoquantal, and the effect of no more than one of them may be recorded during a given EJP, transmitter release will not appear to be multiquantal. The EJP is graded with stimulation strength because at low strengths fewer intramural axons, and hence varicosities, are activated than at higher strengths, leading to different average levels of depolarization in the tissue. Hence, the EJP can be as small as the smallest SEJP, or as large as the largest, depending upon the stimulation intensity. For a more detailed account of this somewhat unusual quantal relation, the interested reader is referred to the relevant papers and reviews[30,31,41–43,46].

Summary

To summarize, theoretical predictions and experimental results are now in good agreement about the factors governing junction potential time courses in smooth muscle. Extracellular recordings have made it possible to measure the kinetics of junction currents. Simultaneous intracellular and extracellular recordings have established that whereas in the case of the SEJP membrane potential change follows the time course of underlying current, in the case of the EJP it is much more prolonged than the current. Spontaneous and evoked junction currents are similar in time course. These results can be explained in terms of the syncytial nature of smooth muscle by postulating redistribution of charge in a three-dimensional resistive network in the syncytium during the SEJP and in an approximately isopotential resistive–capacitative network during the EJP.

Support to this scheme has been lent by studies on other kinds of smooth muscle, notably vascular smooth muscle, in one particularly interesting case by demonstrating a corollary to the hypothesis. Hirst and Neild[47,48] described a guinea pig submucosal arteriolar preparation which was just one smooth muscle cell layer thick. By using short segments of arteriolar branches which were less than 0.35 length constants long, they showed that the arteriole behaved as a one-dimensional cable even for intracellular current injection. Under these conditions, the time constant of decay of not only the EJP[47] but also the SEJP[48] was shown to be similar in value to τ_m, and the time courses of the EJP and the SEJP were identical. Hirst and Neild[47] also estimated that the membrane currents underlying both the EJP and the SEJP were relatively brief. Hence, when an otherwise three-dimensional syncytium is rendered – by fortuitous physiological arrangement – unidimensional and cable-like, its electrical behaviour becomes similar to that of a skeletal muscle cell. Finkel *et al.*[49] succeeded in voltage clamping this one-dimensional arteriolar preparation with a single-microelectrode voltage clamp system and corroborated these suggestions. It is interesting that the time course of the conductance change in this vascular smooth muscle resembles the time course of the SEJP in the vas deferens[47–50]. In other vascular smooth muscle, where three-dimensional syncytial properties are exhibited, tissue electrical properties resemble those of the vas deferens[51].

Conclusions

In this review I have outlined the often tortuous development of ideas on smooth muscle electrical properties during neurotransmission. It is noteworthy at every stage that the methods adopted for determination of electrical properties have strongly influenced our theoretical picture. Thus, although we now have a hypothesis that consistently explains many observations and may have heuristic value, it might subsequently require modification in the face of new observations obtained with more refined techniques. Additional questions that require attention are:

(i) In some smooth muscles evidence from different lines of experimentation is in conflict regarding whether the cells are electrically coupled. In the vas deferens of the rat and the mouse it has not been possible to demonstrate cable-like properties using the partition stimulation method[36], indicating restricted or absent intercellular current flow, at least in the longitudinal direction. Yet, in these tissues the relationship between the time courses of the junction potentials and the currents is as outlined above[44], indicating effective electrical coupling. Such discrepancies need to be resolved.

(ii) What are the properties, in terms of unitary conductance and mean open time, of the neurotransmitter-activated ion channels which underlie the development of the SEJP and the EJP? Patch clamp studies have so far failed to provide the answer, as only whole-cell recordings have been possible[12]. An alternative approach to the problem might be to carry out fluctuation analysis of transmitter-activated membrane noise[5,37], which would provide indirectly, but reliably, the information of interest.

(iii) Not all EJPs have simple time courses. In many cells their decaying phases can be complex, being described by more than one time constant of decay[52], and one of these phases may be briefer than τ_m. Theoretical studies need to be done on how such EJPs might arise. This may perhaps best be begun by considering simple equivalent circuits for the tissue, intermediate in complexity between the three-dimensional syncytial and the isopotential cases, and estimating the voltage responses in these[53].

(iv) The identity of the subcellular structures that allow intercellular electrical coupling in smooth muscle is controversial. In other syncytial tissues, the structure generally accepted to underlie coupling is the 'gap junction'[54]. However, in the case of smooth muscle the evidence for the existence of gap junctions between cells is equivocal, as is their function when present[54,55]. It will be interesting to see whether a morphological substrate is soon established for the electrical coupling that is so strikingly evident functionally and that plays such an important role in determining tissue electrical properties under different conditions.

1 Bennett, M R , *Philos. Trans. R Soc London*, 1973, **B265**, 25–34
2 Tomita, T , *Prog. Biophys. Mol. Biol* , 1975, **30**, 185–203
3 Katz, B , *Nerve, Muscle, and Synapse*, Mc-Graw Hill, New York, 1966
4 Prior, C , Dempster, J. and Marshall, I G , *J Pharmacol. Toxicol Methods*, 1993, **30**, 1–17.
5. Nicholls, J G., Martin, A R. and Wallace, B , *From Neuron to Brain*, 3rd edn, Sinauer, Sunderland, 1992.
6. Unwin, N., *Nature*, 1995, **373**, 37–43
7. Brock, J. A C. and Cunnane, T. C., *Nature*, 1987, **326**, 605–607.
8 Cunnane, T C. and Manchanda, R., *Neuroscience*, 1989, **30**, 563–575
9. Huizinga, J. D , *Can. J. Physiol. Pharmacol* , 1991, **69**, 1133–1142
10 Tomita, T., *J. Theor. Biol.*, 1966, **12**, 216–227.
11. Tomita, T., *J. Physiol.*, 1967, **189**, 163–176.
12. Tomita, T., *Jpn J. Physiol.*, 1992, **58**, 1P–6P
13 Purves, R D , *J. Theor. Biol* , 1976, **60**, 147–162.
14. Burnstock, G , *Experientia*, 1985, **41**, 869–874.
15. Bozler, E., *Philos Trans. R. Soc. London*, 1973, **B265**, 3–6.
16. Abe, Y. and Tomita, T., *J. Physiol.*, 1968, **196**, 87–100
17 Tomita, T., in *Smooth Muscle* (eds. Bülbring, E., Brading, A. F., Jones, A. and Tomita, T.), Edward Arnold, London, 1970, pp. 197–243.
18 Lavidis, N. A. and Bennett, M. R., *J. Auto. Ner. Sys.*, 1993, **45**, 87–100.
19. Hodgkin, A L. and Rushton, W. A. H , *Proc. R Soc London*, 1946, **B133**, 444–479
20 Katz, B , *Proc. R. Soc. London*, 1948, **B135**, 506–534
21 Jack, J. J. B., Noble, D. and Tsien, R. W , *Electric Current Flow in Excitable Cells*, Oxford University Press, Oxford, 1983.
22. Fatt, P. and Katz, B., *J. Physiol.*, 1951, **115**, 320–370.
23. Jack, J J B. and Redman, S. J., *J. Physiol.*, 1971, **215**, 321–352
24 Gage, P. W., *Physiol Rev.*, 1976, **56**, 177–247.
25. Faber, D. S and Korn, H., *Science*, 1980, **208**, 612–614
26. Kuriyama, H., *J Physiol* , 1964, **175**, 211–230.
27. Bywater, R. A R and Taylor, G. W., *J. Physiol.*, 1980, **300**, 303–316
28 Burnstock, G and Holman, M E , *J. Physiol.*, 1961, **155**, 115–133
29 Burnstock, G. and Holman, M E , *J Physiol.*, 1962, **160**, 446–460
30 Blakeley, A G H and Cunnane, T. C , *J. Physiol* , 1979, **296**, 85–96
31. Cunnane, T. C and Stjärne, L., *Neuroscience*, 1984, **13**, 1–20
32. Del Castillo, J and Katz, B , *J. Physiol* , 1954, **124**, 560–573
33 Cunnane, T C and Manchanda, R , *Neuroscience*, 1990, **37**, 507–516
34 Hashimoto, Y , Holman, M E. and Tille, J , *J Physiol* , 1966, **186**, 27–41.
35 Bennett, M R , *J. Gen Physiol* , 1967, **50**, 2459–2470.
36 Holman, M E , Taylor, G S and Tomita, T., *J. Physiol* , 1977, **266**, 751–764
37. Anderson, C. R and Stevens, C F , *J Physiol* , 1973, **235**, 655–693
38 Bolton, T B , Tomita, T and Vassort, G , in *Smooth Muscle An Assessment of Current Knowledge* (eds Bulbring, E , Brading, A. F., Jones, A and Tomita, T), Edward Arnold, London, 1981, pp 47–63.
39. Del Castillo, J and Katz, B., *J. Physiol.*, 1956, **132**, 630–649
40 Holman, M E , in *Smooth Muscle* (eds Bülbring, B , Brading, A F , Jones, A and Tomita, T.), Edward Arnold, London, 1970, pp 244–288
41. Brock, J. A. C. and Cunnane, T C , *J. Physiol.*, 1988, **399**, 607–632.
42 Åstrand, P., Brock, J C. and Cunnane, T. C., *J. Physiol* , 1988, **401**, 657–670
43. Lavidis, N. A. and Bennett, M. R., *J Physiol* , 1992, **454**, 9–26
44. Manchanda, R , D. Phil Thesis, University of Oxford, 1989
45 Bennett, M R., Gibson, W. G and Poznanski, R. R , *Philos. Trans R. Soc London*, 1993, **B342**, 89–99
46. Stjärne, L , Åstrand, P., Bao, J-X , Gonon, F., Msghina, M. and Stjärne, E , in *Molecular and Cellular Mechanisms of Neurotransmitter Release* (eds Stjärne, L., Greengard, P., Grillner, S , Hökfelt, T and Ottoson, D), Raven Press, New York, 1994, pp 461–496
47 Hirst, G. D S and Neild, T. O , *J. Physiol* , 1978, **280**, 87–104.
48 Hirst, G. D S. and Neild, T. O., *J. Physiol.*, 1980, **303**, 43–60.
49. Finkel, A. S., Hirst, G. D S. and van Helden, D F., *J Physiol* , 1984, **351**, 87–98
50. Hirst, G. D. S , De Gleria, S. and van Helden, D. F., *Experientia*, 1985, **41**, 874–879
51 Cassell, J F , McLachlan, E. and Sittiracha, T., *J. Physiol* , 1988, **345**, 31–49.
52 Jobling, P. and McLachlan, E. M , *J Physiol* , 1992, **454**, 83–105.
53. Manchanda, R , *Electro and Magneto Biol* , 1994, **13**, 99–106.
54 Huizinga, J D , Liu, L W. C., Blennerhassett, M G., Thunerberg, L and Molleman, A , *Experientia*, 1992, **48**, 932–941
55. Garfield, R E , Thilander, G and Blennerhassett, M G., *Can J Physiol Pharmacol* , 1992, **70**, 481–490

Received 31 January 1995; revised accepted 21 April 1995