Quantal depolarizations at the cellular and molecular level in smooth muscle

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At synapses, signal transmission takes place by the release, from the presynaptic neuronal terminal, of a chemical substance, the neurotransmitter, which then acts upon the postsynaptic cell. Two features of synaptic transmission are well recognized. (i) The neurotransmitter is stored and released in multimolecular packets which are seen in electron micrographs as spherical transmitter-containing vesicles present at the nerve terminal. (ii) The neurotransmitter molecules act by binding to receptors embedded in the postsynaptic membrane, which then mediate the response[1,2]. Often the response is a transient change in membrane potential of the postsynaptic cell (the synaptic or junction potential). In excitatory transmission, transmitter-activated depolarization results from transmembrane ionic fluxes induced by the activation of ionic channels directly or indirectly associated with the receptors[2,3].

Quantization of processes involved in neuromuscular transmission occurs at atleast two levels – the cellular and the molecular – as observed at some of the well-studied synapses, such as the skeletal neuromuscular junction. While at the molecular level, the irreducible quantal response is the 'elementary' or 'single-channel' depolarization (or underlying membrane current) resulting from the activation of a single receptor-operated ion channel[4,5], at the cellular level, the quantal response is the summed multi-channel depolarization (or current) produced by the transmitter contents of a single vesicle, i.e. the spontaneously occurring miniature end plate potentials (MEPPs)[6,7]. Classically, it is this event at the cellular level that has gained recognition as the 'quantal' depolarization. According to the 'quantal hypothesis', MEPPs represent irreducible units of transmitter action, this has been established by comparison of their amplitudes with the nerve-stimulation-evoked depolarizations (EPPs). The crucial observation is that the preferred peak amplitudes of the EPPs, particularly under conditions of low probability of transmitter release, are integral multiples of the unimodally distributed mean amplitude of the MEPPs[6,8]. Thus the evoked event is taken to be the summation of the effects of one to several quanta. A similar quantal relationship between spontaneous and evoked synaptic potentials has been shown to exist at several synapses[7].

Quantal operation at the molecular level is substantiated by the observation at a variety of synapses that the mean open time (θ) of single receptor operated channels (ROCs) corresponds closely to the time constant of decay (τ_{decay}) of the quantal junction current[4,5,9]. Given this correspondence, it can be shown that an exponential distribution about the mean open time of channel provides a cellular-level current that mimics the quantal current[10].

In comparison with the detailed information now available on the quantal features of transmission at several synapses, our knowledge on these fronts about transmission at autonomic neuromuscular junctions (ANJs) in smooth muscle organs is relatively limited. A representative example is the mammalian vas deferens, where it is still unclear whether the spontaneously occurring depolarization (spontaneous excitatory junction potential, SEJP), which is the equivalent of the MEPP, is the basic quantal unit of the stimulation-evoked EJP[11,12]. The following features of these junction potentials contribute to the uncertainty: (1) the peak amplitudes of SEJPs and EJPs fall in the same range; the EJP is not observed to be an integral multiple of the SEJP; (2) the SEJP and EJP have widely differing time courses, the EJP being up to ten times more prolonged than the SEJP[11,12]. On both grounds, the construction of the EJP in terms of a multi-quantal SEJP is not immediately apparent.

At the molecular level too, information about the elementary or single-channel depolarizations that underlie the cellular quantal event (the SEJP) is limited. The properties of single channels can be observed directly, using the patch clamp technique[9], or indirectly inferred, using the technique of fluctuation analysis[13]. Neither of these techniques has been applied to the elucidation of ROC properties in smooth muscle.

Recent experiments in our laboratory have shed light on the quantal features of transmission in smooth muscle, at the cellular level as well as the molecular level. At the cellular level, the experiments which have their basis in the rationale, that since syncytial behaviour may obscure quantal features of transmission (see Discussion), some insight might be gained by functionally uncoupling smooth muscle cells from one another. A chemical agent that has recently been reported to exert such an action, is the aliphatic alcohol, heptanol[14]. The effects of heptanol

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on the electrophysiological properties of a conveniently explored organ, the vas deferens, were therefore investigated. We found that heptanol reveals quantal depolarizations that underlie the EJPs of the guinea pig vas deferens, and that these are identical to the SEJPs. Information has also been acquired, by the technique of fluctuation analysis, about the quantal deplorizations at the molecular level that underlie the cellular quantal depolarizations, the SEJPs. In the guinea pig vas deferens, which receives a purely sympathetic motor innervation, the postjunctional electrical events (EJPs and SEJPs) are probably produced by adenosine 5′-triphosphate (ATP), released as a neurotransmitter from the sympathetic axons[15]. Knowledge of the syncytial electrical behaviour of the smooth muscle has enabled us to use the results of ATP-induced voltage fluctuations, to infer single-channel ROC properties in this tissue, and to show that these properties are consistent with their being the electrical 'building blocks' of the SEJPs.

Methods

Vasa deferentia were obtained from male guinea pigs weighing 350–450 g, sacrificed by stunning and exsanguination. The abdomen was opened by a midline incision and vasa on either sides were exposed by pulling out the testes from the scrotal sacs. The vasa were dissected out along with the innervating hypogastric nerve. The organ was pinned out under slight tension on a piece of silicone rubber lining the base of a 5 ml perspex dual chamber bath.

The tissue was continuously bathed in Krebs solution at 37°C, which dripped into the bath at 2–3 ml/min under the influence of gravity and was removed by suction. The Krebs solution had the following composition: (mM) NaCl 118.4, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, NaH_2PO_4 0.4, glucose 11.1. It was continuously bubbled with carbogen gas ($O_2 : CO_2 :: 95 : 5$) to maintain its pH at 7.3–7.4. The hypogastric nerve was stimulated using a pair of Ag/AgCl ring electrodes to obtain evoked potentials and currents.

Electrical recordings

The details of the methods used for electrical recordings have been provided elsewhere[16]. Briefly, changes in transmembrane potential (EJPs and SEJPs) were recorded intracellulary, using glass microelectrodes with tip resistance of 50–100 MΩ. The microelectrodes were filled with 3 M KCl and connected to the headstage of an electrometer (IE201, Warner Instruments, USA), the signals being low-pass filtered (– 3 dB cutoff at 1 kHz).

To elicit ATP potentials for fluctuation analysis, solutions of ATP in Krebs (concentration 10–100 μM) were applied on the surgically cleaned surface of the vas through a microelectrode (tip diameter 40–50 μm), using pressure pulses delivered to the electrode by an application device (Picospritzer, General Valve Co, USA). The Picospritzer was capable of delivering pulses whose width and pressure could be varied as required (see Results for values employed). Electrical responses to ATP application were recorded using intracellular, microelectrodes inserted in cells at a distance of 0.5–3 mm from the ATP application electrode. Electrophysiological signals were displayed on an oscilloscope and stored on a DAT recorder (DTR 1204, Biologic, France) for future analysis.

Data analysis

Signals played back on the analogue output channel of the DAT recorder were amplified, if necessary, and digitized using a PC–AT interfaced A/D card (PCL 209, Dynalog Microsystems Ltd, Mumbai). Data were subsequently analysed using appropriate software routines in the programs SCAN (for junction potentials) or SPAN (for ATP-evoked membrane potential fluctuations) available as part of the Strathclyde Electrophysiology software (SES) suite[17] (supplied by Dr J. Dempster, Strathclyde University, UK).

Results

Quantal depolarizations underlying the EJP

When the presumptive gap junction uncoupler heptanol was superfused over the vas deferens isolated from guinea pig, intracellularly-recorded stimulation-evoked EJPs were affected in two ways. In about 75% of the cells examined, EJPs were fully and reversibly abolished, as reported earlier (Figure 1A; ref. 16). There was no concomitant change in the resting membrane potential, indicating that heptanol did not exert non-specific electrical actions on the resting cell membrane. The time course of the EJP did not change during its suppression, as seen in Figure 1A while the EJPs were suppressed or otherwise altered in the presence of heptanol, SEJPs were unaffected in their amplitudes as well as in their time courses[16].

In the remaining cells monitored (~ 25%), although the EJP was suppressed by heptanol, a kind of stimulus-locked depolarization that has not been reported earlier continued to occur in the presence of heptanol. For this discussion, we shall refer to the usual prolonged depolarization of the EJP as the 'background' depolarization to distinguish it from the novel depolarizations observed following heptanol action. In these cells, once the background EJPs were suppressed to less than about 50%

of their control amplitudes by heptanol (Figure 1B *b,d*), short-duration stimulus-locked depolarizations occurred intermittently, at the same latency as the EJP. When virtually no background depolarization of the EJP remained (< 1 mV), the resolution of the transient stimulus-locked depolarizations improved, and a conspicuous resemblance to SEJPs emerged (Figure 1B *f*). For this reason and others to be elaborated below, these events will henceforth be referred to as 'quantal EJPs' (QEJPs). Examples of the short-duration stimulus-locked QEJPs are provided in Figure 1B *b,d,f*, from a cell whose EJPs were suppressed by heptanol almost completely.

Several other properties of QEJPs, viz. latency, frequency of occurrence, amplitudes and time courses, were also found to be consistent with the idea that these signals may be the quantal depolarizations underlying EJPs. A description of these properties is given below.

(i) *Latency.* Following the stimulus, QEJPs commenced over a narrow latency band that fell within the wider band of EJP latencies. This suggests that QEJPs perhaps reflect responses generated by a subset of the prejunctional axons that generate the EJP.

(ii) *Intermittence.* QEJPs occurred intermittently, i.e. not every stimulus delivered to the hypogastric nerve succeeded in eliciting a QEJP (Figure 2 *a*). The probability of occurrence of QEJPs varied between 0.01 and 0.16 in different cells. It should be noted that these levels of intermittence fall within the range of two other electrophysiological signals reported earlier to represent secretory activity from a local population of release sites, i.e. 'discrete events'[18], and extracellularly recorded excitatory junction currents (EJCs)[19].

(iv) *Configuration.* QEJPs varied in amplitude and time course from one event to another (Figure 1B *f*). Occasionally, however, one QEJP was followed within a few stimuli by an apparently identical one. The rise times, time constants of decay and total durations of QEJPs were not significantly different compared to those of SEJPs recorded in the same cell (Figure 2 *b*, Table 1).

Certain QEJPs could also be matched precisely with SEJPs occurring in the same cell. Figure 2 *b* shows a selected QEJP and SEJP recorded from the same cell. It is evident that in the configuration of rising and decaying phases, the QEJP is virtually identical to the SEJP. The observation of QEJPs in the presence of heptanol indicates that the fundamental depolarization underlying the EJP in smooth muscle may be identical to the SEJP.

As with intermittency, the features of (i) temporally closely spaced occurrence of the same QEJP, and (ii) accurate match between an evoked and a spontaneous event, are characteristic also of discrete events (DEs) and evoked excitatory junction currents (EJCs). These latter signals are believed to represent quantal transmitter release events[18,19], hence our choice of the term 'quantal EJPs' to describe the rapid evoked depolarizations observed in the presence of heptanol.

Quantal depolarizations underlying the SEJP: Analysis of ATP-evoked membrane potential fluctuations

ATP potentials, i.e. EJP-like transient depolarizations due to extraneous application of ATP[20,21] were evoked following focal application of ATP, using pressure pulses delivered to the ATP-containing microelectrode whose tip was positioned close to the surface of the vas (Figure 3). The properties of the potentials, e.g. rise time, amplitude and decay, depended on a range of factors, viz. (i) the pulse parameters, i.e. pulse duration and

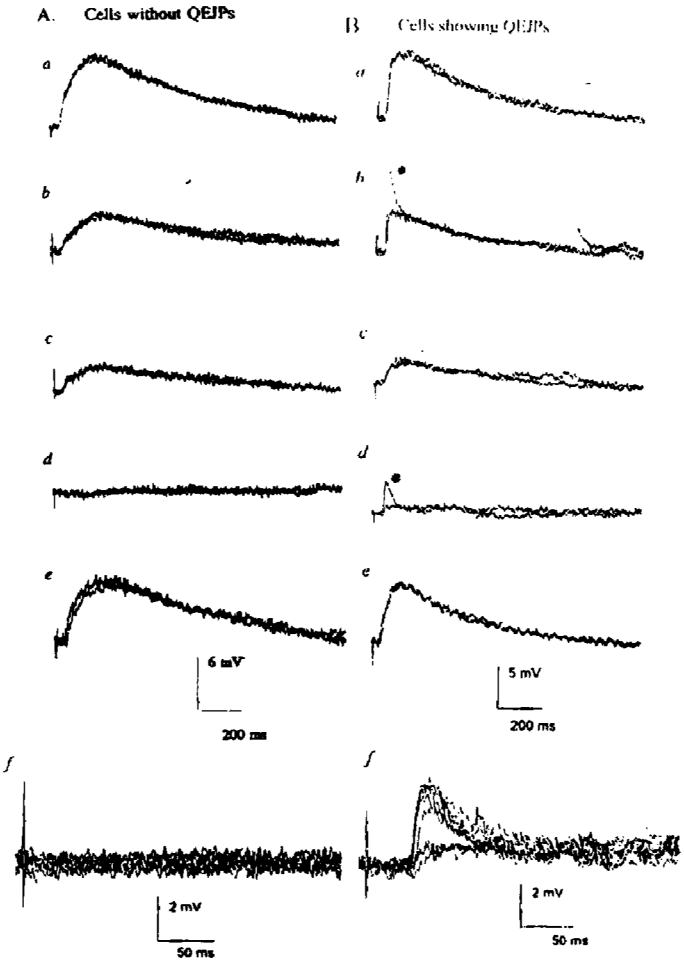

Figure 1. Suppression by heptanol of EJPs, unaccompanied (A) or accompanied (B) by the unmasking of rapid evoked depolarizations (QEJPS: asterisks). Records in A and in B are from a single cell. Each panel has 3-4 successive EJPs superimposed. All recordings in this and subsequent figures were made from the guinea pig vas deferens.

pressure; (ii) the distance of the application electrode from the recording electrode or from the surface of the tissue; and (iii) the concentration of the ATP used.

In order to infer properties of ATP-activated receptor-operated channels (ROCs) at the single channel level from membrane potential fluctuations evoked by ATP, it was necessary to obtain plateau-like depolarizations rather than the transient depolarizations of the ATP potentials[22]. Pilot studies were performed to arrive at optimal conditions of ATP application under which such steady depolarizations could be obtained. Under appropriate conditions, it was found that it was necessary to use application over several seconds, and at lower concentrations of ATP than used for EJP-like ATP potentials. As illustrated in Figure 4B, steady application of ATP for 15–30 sec (10^{-5} M) gave rise to a plateau-like depolarization. This depolarization on amplification revealed fluctuations that were noticeably different compared to the background noise seen either before (Figure 4A *b*) or after (Figure 4D *b*) the application of ATP. These fluctuations are thought to reflect stochastic variation in the number of open channels about a mean level[13].

The power spectrum of such potential fluctuations has the form of a Lorentzian function characterized by a corner frequency f_c, at which the spectral density drops to half of its zero frequency asymptote. Corner frequency f_c is related to the mean open time of activated channels as $f_c = 1/(2\pi\theta)$, as dictated by the relation

$$S_{vv}(f) = \frac{2V_m a\theta}{1+(2\pi f\theta)^2},$$

where $S_{vv}(f)$ is the autospectral density function of the membrane voltage; V_m the steady-state (d.c. level) membrane depolarization; a the amplitude of the 'shot' depolarization.

This enables estimation of θ (ref. 23). Similar analysis of the ATP-induced fluctuations (Figure 5) after subtracting the background noise provided a value of θ of 45.7 ± 4.5 ms (mean $\pm$ SEm, $n = 5$). The time constant

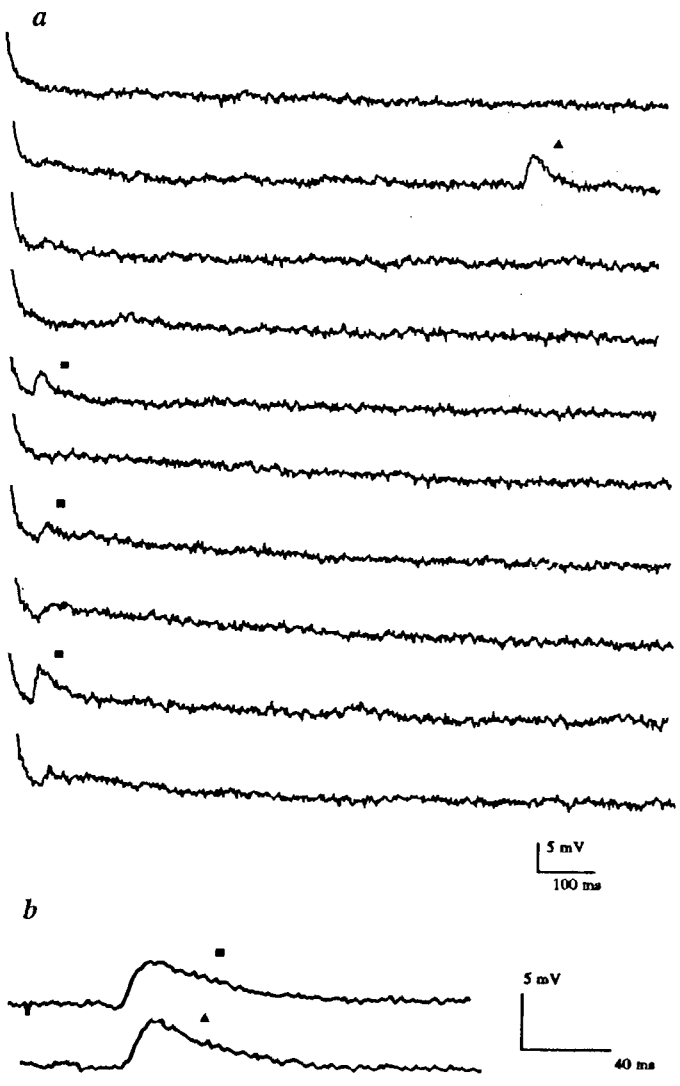

Figure 2. *a*, Series of evoked events following heptanol action, showing the absence of EJPs and the intermittent occurrence of QEJPs. *b*, An SEJP and a QEJP recorded from the same cell, selected to show the close correspondence in amplitudes and time courses. ■, QEJPs; ▲, SEJPs.

Table 1. Temporal properties of SEJPs and QEJPs compared statistically (Student's *t* test)

	Rise time (ms)	T_{decay} (ms)	Duration (ms)
SEJPs	11.2 ± 1.1 (19)	30.2 ± 1.3 (9)	85.1 ± 3.8 (16)
QEJPs	13.2 ± 0.8 (14)	28.0 ± 2.9 (10)	95.6 ± 5.3 (12)
P	0.178	0.508	0.111

Data are mean ± SEm (no. of observations). $P > 0.1$ indicates no significant difference.

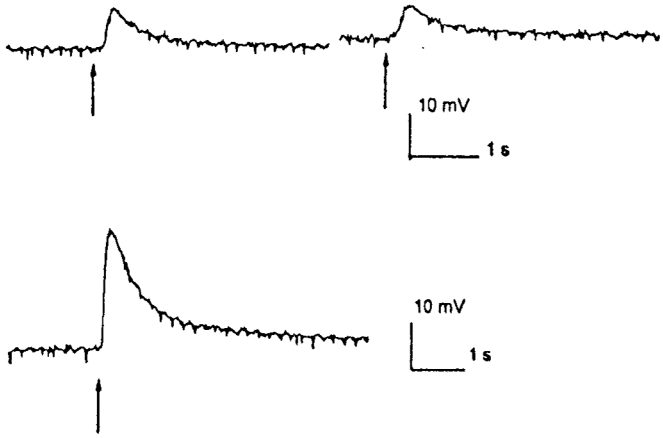

Figure 3. EJP-like ATP potentials (potentials due to brief local application of 100 µM ATP). Potentials in the top and bottom panels are from two different cells. Arrow indicates instant of application of ATP (pulse duration 20 ms).

of decay of SEJPs observed under similar conditions was 37.3 ± 10.9 ms, $n = 23$. This value is in reasonable agreement with the mean open time of ATP-activated channels obtained from fluctuation analysis.

To test the possibility that these fluctuations might be artifacts produced due to the methodology of pressure application, Krebs solution alone was applied through the external microelectrode in a fashion similar to that for ATP. At low application pressure, this procedure did not evoke noticeable membrane potential fluctuations. At application pressures much higher than those used for ATP, membrane potential fluctuations were produced, but these were grossly dissimilar to those evoked by ATP. These observations indicate that the fluctuations observed with ATP biological in origin, were probably caused by the action of ATP on P_{2x} purinoceptors.

Discussion

In previous work, although a depolarization of the time course of the SEJP (whose τ_{decay} is 25–50 ms) has been suggested to be the quantal depolarization underlying the EJP (τ_{decay} 200–300 ms) (refs 24, 25), this relation has never been directly verified. Furthermore, it has not been certain that the neurotransmitter suggested to mediate the junction potentials in the vas deferens, ATP, generates quantal depolarizations at the single-channel level whose temporal characteristics are consistent with its mediation of the SEJP.

The present observations throw light on both these quantal aspects of neuromuscular transmission in smooth muscle. The first quantal depolarization of interest is that which underlies the stimulation-evoked EJP, by the action of heptanol. The second is the single-channel associated depolarization underlying the 'quantal EJP', as revealed by the steady-state electrical action of ATP on the muscle membrane, and its relation to the neurogenic SEJP.

Quantal EJPs

The quantal hypothesis of neurotransmission at the cellular level states that synaptic neurotransmitters are stored and released in the form of irreducible multimolecular packets, in the form of the presynaptic storage vesicles observed under the electron microscope. Vesicular secretion neurotransmitter can be either spontaneous or stimulus-dependent, being evoked in the latter case by invasion of the presynaptic axon by an active impulse. Spontaneous release is usually monoquantal and evoked, multiquantal[7]. The electrophysiological evidence for this hypothesis is that the neurotransmitter activated postjunctional electrical events can be seen to be multiples of a basic unit; the spontaneously produced membrane potential change being the basic unit and the evoked unit, usually a multiple of this.

At the neuromuscular junction in smooth muscle, events analogous to the MEPPs and EPPs are the spontaneous and stimulation-evoked excitatory junction potentials (SEJPs and EJPs)[26,27] respectively. However, these junction potentials do not show a quantal relationship similar to that which holds between MEPPs and EPPs. The reasons for this are: 1) the EJP has a longer time course, compared to the SEJp; 2) the peak amplitude of an SEJP can be as large as the EJP; 3)

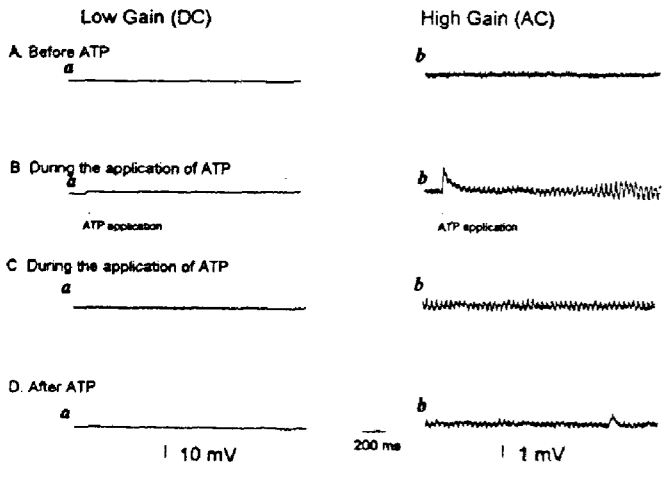

Figure 4. Membrane potential fluctuations caused by steady application of 10 μM ATP. While the left panel (traces labelled *a*) shows low gain, DC-coupled membrane potential traces, the right panel (traces labelled *b*) shows membrane potential noise obtained by AC coupling and amplifying the corresponding DC traces. Onset of application indicated by an arrow in B.

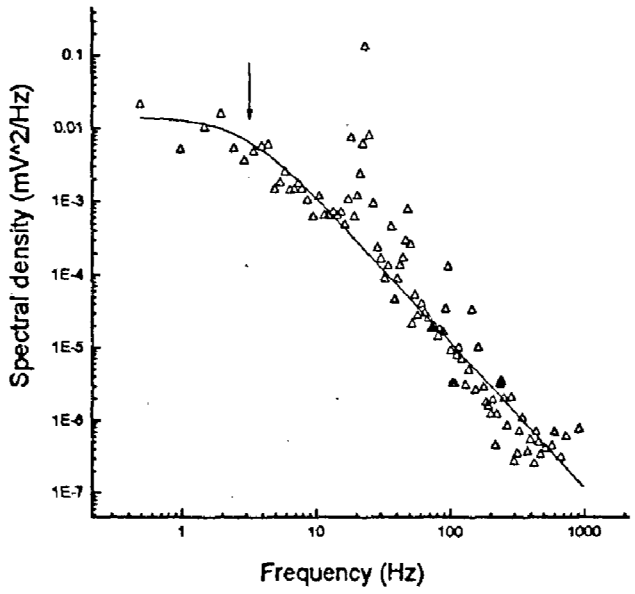

Figure 5. Lorentzian plot for ATP-induced membrane potential fluctuations. Corner frequency (f_c) marked by the arrow.

the amplitude histogram of SEJPs has been found to be skewed towards the low amplitude events[27] as opposed to the Gaussian distribution of MEPPs (ref. 1). All these features of neurotransmission at the autonomic neuromuscular junction, which apparently militate against the quantal hypothesis, are thought mainly to be due to the properties imparted to the tissue by virtue of intercellular electrical coupling[12,28,29]. Therefore, the effects of a putative inhibitor of intercellular electrical coupling, heptanol, on junction potentials were explored.

The effects of heptanol on EJPs seem to provide further insight on the generation of the EJP. The fundamental quantal contributions to the EJP have been suggested to be identical to SEJPs, based on various lines of argument[24,25,30,31]. However, evoked quantal electrical events resembling SEJPs have never been observed directly, since they are submerged in the general syncytial depolarization of the EJP. Our results show that these quantal depolarizations (QEJPs) can be detected following the application of heptanol. Since various properties of these QEJPs, e.g. range of amplitudes and time courses, are essentially the same as those of SEJPs (Figures 1 and 2), this corroborates the suggestion that the basic evoked quantal depolarization is an SEJP-like event.

If the QEJP represents the true quantal event underlying the EJP, it becomes necessary to consider how the action of heptanol should result in the appearance of this signal.

Heptanol is known to specifically block gap junctional channels, thus uncoupling syncytial cells from one another electrically[14,32–34]. It has been reported to block intercellular coupling in a variety of cell types at concentrations between 1 and 5.6 mM (refs 14, 32, 34, 35, 36). In smooth muscle, it seems to affect contractility by a specific effect on uncoupling at concentrations ≤ 2 mM (ref. 14).

A specific block of gap junctional communication by heptanol may allow the resolution of transmitter action at individual neuromuscular junctions as follows. When a cell or group of cells has been uncoupled from its neighbours, depolarizations generated remotely, that are normally propagated passively to that cell, may no longer be recorded in it. The underlying assumption is that the EJP is normally composed of a QEJP-like signal generated locally in the tissue superposed with a longer-lasting background depolarization generated at a larger distance.

It may be suggested that the background depolarization of the EJP is removed during the action of heptanol, leaving behind only locally generated quantal depolarizations caused by activation of nearby CCVs. However such an explanation must at present remain speculative because the exact mechanism of action of heptanol at these neuromuscular junctions is yet to be explored fully. Certain observations are inconsistent with the idea that heptanol blocks intercellular electrical coupling at the level of individual smooth muscle cells. Had this been the case, SEJPs would have been expected to be prolonged in time course, and the frequency of their observation reduced, effects that are not observed experimentally. A possible explanation is that heptanol may be uncoupling bundles of cells[37] from one another electrically, which may explain the range of observations. Studies towards clarifying the precise mode of action of heptanol are currently in progress.

ATP-induced membrane potential fluctuations

The relation between the quantal depolarization at the cellular level (the SEJP following spontaneous neurotransmitter release and the QEJP following evoked release), and the underlying microphyiological event, i.e. depolarization due to the opening of a single transmitter-activated channel has not previously been explored. By employing the technique of fluctuation or 'noise' analysis, which indirectly provides estimates of channel opening kinetics and amplitude properties[4,13], we are now able to comment on this relation.

In the present studies ATP-induced fluctuations abounding a mean level of membrane depolarization have been analysed. It should be noted, however, that information about channel activation in other tissues is usually obtained from the measurement of membrane *current* noise and underlying conductance change, since this reflects ion channel activation directly. The properties of membrane *potential* fluctuations, on the other hand, like those of any other variation in membrane potential, are determined not just by the underlying current time course but also by the passive electrical properties of the membrane[23]. These determine the impulse response of the cell or group of cells investigated to the neurotransmitter-activated input (membrane current). In most cells, membrane time constant is substantially longer than the duration of neurotransmitter activated conductance change. Therefore analysis of membrane potential fluctuations for temporal properties generally provides information about the time constant of the cell membrane rather than the activation of the underlying ionic channels[23,38].

The fundamental electrical properties of smooth muscle, however, are such as to allow us to use membrane potential fluctuations to infer channel properties directly. The complication introduced by the possible contribution of membrane time constant is obviated in smooth muscle, owing to the electrical behaviour of this tissue as a three-dimensional syncytium. In syncytial tissue, the low-resistance shunts offered by extensive intercellular coupling pathways allow the cellular matrix to behave electrically as an almost purely resistive medium for the dissipation of neurotransmitter activated membrane

current[28,29]. Since potential change across a purely resistive impedance follows the time course of current flowing through it, membrane potential change in a well-coupled syncytium should follow the time course of its underlying current, being no more prolonged than the latter. This is demonstrated convincingly by the fact that for the SEJP, which is produced by focal current injection at a spatial point in the syncytium, the membrane potential change follows precisely the time course of its underlying current (the SEJC) (refs 25, 31); hence the membrane time constant does not influence the discharge of current[12]. Similar conclusions arise from an analysis of EJP-like ATP potentials and their underlying currents evoked in the vas deferens[25].

It may therefore be suggested that in syncytial smooth muscle the membrane potential changes produced by individual *channel* activations would similarly reflect the kinetics of the underlying channel conductance change. Hence analysis of membrane potential fluctuations in smooth muscle should provide reliable information about the kinetics of channel activation instead of reflecting membrane time constant.

The mean open time θ of ATP-activated channels, derived from fluctuation analysis, has been found in our studies to be comparable with the decay time constant of SEJPs. BY analogy with the findings at the skeletal nmj[23] and other synapses[38–40], it can be suggested that the quantal depolarization of smooth muscle (the SEJP) may be composed of the transient opening of population of ATP-gated channels with $\theta \approx 40$ ms with the open times exponentially distributed about this mean (Figure 6 *b*).

From preliminary analysis of spectra we estimate the amplitude of the elementary depolarization (V_{el}) to be ~ 20–30 μV. This suggests an estimate of the number of ATP-activated channels underlying 3–10 mV SEJPs, to be approximately 100–500. Assuming a value for input impedance (R_{in}) of ~ 25 MΩ, we further suggest the amplitude of the unitary current (i_{el}) necessary to produce an elementary depolarization 25 μV in amplitude to be about $(25 \times 10^{-6}\ \text{V})/(25 \times 10^{6}\ \Omega)\ \text{A} \approx 1$ pA. With a reversal potential V_{rev} for the ATP-activated current close to 0 mV (refs 41, 42) and membrane potential of – 60 mV, the elementary conductance change γ_{el} would be:

$$\gamma_{el} = I_{el}/(V_m - V_{rev}) = 10^{-12}\ \text{A}/(60 \times 10^{-3})\ V \approx 16\ \text{pS}.$$

It is interesting that the values of i_{el} and γ_{el} so obtained are of the same order as those inferred[4] or measured[5] for the ACh-activated nicotinic receptor-ion channel at the skeletal muscle and end plate.

It may be noted that although mimicry of EJPs on external application of ATP has been demonstrated[20], mimicry of SEJPs using similar methods has not been demonstrated, mainly because the exact conditions under which a SEJP is generated (close-contact quantal release from a neuronal varicosity) cannot be replicated experimentally. Extraneous addition of ATP, even in the form of brief pulses (6–8 ms) applied directly on the tissue, appears to result in a number of cells being simultaneously depolarized. Since the application of ATP affects an area much larger than that of a single cell, a significant volume of the tissue is probably rendered isopotential, and an EJP-like event is consequently generated[25]. Our results, which show that ATP may induce individual channel activation events consistent with its mediation of the SEJP, consequently serve also to strengthen the hypothesis that ATP mediates both the evoked and the spontaneous postjunctional potentials in sympathetically innervated mammalian smooth muscle.

Conclusions

Figure 6 summarizes the quantal features of neurotransmission at the autonomic nerve – smooth muscle junction, as inferred from the present investigation. The cellular-level quantal depolarization, the SEJP, is

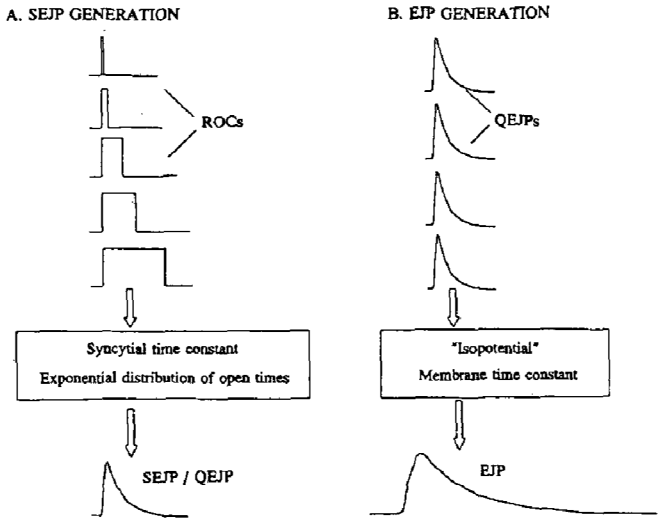

Figure 6. Showing how quantal depolarizations in smooth muscle lead to the eventual generation of the EJP. The panel on the left illustrates how molecular-level quantal depolarizations rectangular in shape can sum to produce a smoothly varying cellular-level quantal event, the SEJP. See text for explanation. This representation is inaccurate in showing the rising phases of the single-channel currents to be precisely aligned. In reality they must be appropriately staggered to produce the gradual rise of the SEJP. Typically the single-channel current amplitude may be a few μV, while the SEJP may rise to a few mV or so. The panel on the right illustrates the integration of QEJPs, which are the evoked analogues of the SEJP, to give rise to the normally recorded EJP. It should be noted that the summation of QEJPs is spatially distributed, and therefore highly nonlinear. Thus the EJP in amplitude is not a multiple of QEJP amplitude, but is prolonged because of the near-isopotential owing to spatially distributed QEJP occurrence (see text for details).

generated by summation of single-channel, molecular depolarizations activated by the contents of a vesicle of neurotransmitter. The open times of these molecular-level quantal depolarizations are exponentially distributed, with the mean open time being similar to the time constant of decay of the SEJP (Figure 6 A).

The nerve-stimulation-evoked EJP is the product of a different kind of integration. SEJP-like events (the QEJPs) occur at individual neuromuscular junction (close-contact varicosity abutting on a smooth muscle cell). A distributed *spatial* summation of these quantal cellular depolarization results in little augmentation of amplitude of depolarization, but a considerable prolongation of its time course (Figure 6 B). The EJP is prolonged because when several QEJPs simultaneously (following nerve stimulation), the smooth muscle syncytium is brought closer to isopotential and neurotransmitter — injected charge is constrained, in each cell, to flow across the membrane impedance. Thus the time constant of decay of the EJP approximates the membrane time constant.

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