THEORETICAL ANALYSIS OF MODE OF HEPTANOL ACTION ON SMOOTH MUSCLE SYNAPTIC POTENTIALS

Rohit Manchanda*, K. Venkateswarlu, S. Sourav, and K. Moudgalya**
School of Biomedical Engineering, **Department of Chemical Engineering,
Indian Institute of Technology-Bombay, Powai, Mumbai - 400 076, INDIA.
*E-mail: rmanch@cc.iitb.ernet.in

ABSTRACT

A theoretical analysis has been undertaken of the synaptic or "junction" potentials of syncytial smooth muscle in order to examine the most likely mode of action of a chemical, 1-heptanol, that has profound effects on neurotransmission in this tissue. The smooth muscle syncytium of the mammalian vas deferens has been modelled as a bidomain electrical grid which allows simulation of the spontaneous and evoked excitatory junction potentials (sEJPs and eEJPs) observed experimentally in this organ. Two hypotheses of heptanol action raised by its effects on the electrical responses of the vas have been examined, (i) inhibition of cell-to-cell electrical coupling in smooth muscle; (ii) inhibition of the stimulation-evoked release of neurotransmitter from the autonomic nerve supply. We find that the range of experimental findings can be explained consistently on the basis of the second, but not the first, hypothesis. Our results thus cast doubt on the current belief that heptanol, at the concentrations used empirically, specifically uncouples smooth muscle cells from one another, and indicate a novel, previously unsuspected biophysical mode of action which merits close scrutiny.

KEYWORDS: Electrical syncytium, quantal depolarizations, smooth muscle, electrical uncoupling, physiological modelling.

I. INTRODUCTION

In syncytial muscles of the body (e.g. smooth and cardiac muscle) electrical continuity between cells is believed to be served by "gap junctions", which are low-impedance proteinic channels spanning the apposed membranes of neighbouring cells [1]. Important clues to the role of syncytial coupling in normal organ function can therefore accrue from studying the effects of disruption of gap junctional function. Recently, the effects of a chemical, 1-heptanol, which is suggested to block gap junctions specifically [1], were explored experimentally on synaptic potentials of the smooth muscle of the guinea-pig vas deferens [2], i.e. the spontaneous and evoked excitatory junction potentials (sEJPs and eEJPs respectively). The salient observations were as follows. (i) In most cells ("passive" cells which do not receive innervation by close-contact varicosities or CCVs), eEJPs were reversibly abolished by 2.0 mM heptanol without change in their time course [2]. (ii) In "active" cells which receive CCVs, a novel kind of stimulus-locked, evoked electrical signal was induced by heptanol. These signals, which replaced the eEJPs, were strikingly similar to sEJPs, and were termed quantal EJPs (qEJPs) [3]. (iii) Heptanol left unaffected the randomly occurring spontaneous EJPs (sEJPs) in all their properties, including time courses and amplitude distribution.

From a qualitative viewpoint, this set of observations could not be consistently explained on the basis of cell-to-cell uncoupling by heptanol [3]. Therefore, it was considered whether alternative modes of action of heptanol might offer a more consistent framework to explain the results. In particular, it was conjectured that heptanol may interfere specifically with stimulation-evoked release of neurotransmitter.

In this paper, we present the results of a theoretical exploration of syncytial electrophysiology, which helps discriminate between the competing hypotheses on hand. Smooth muscle junction potentials have been simulated recently in a discrete model of the syncytium [4]. In the present study we undertook to use this model so as to incorporate the conditions appropriate to each of the hypotheses under examination. We report here the predictions made by the model under conditions of (i) gap junctional inhibition between individual cells throughout the syncytium (Hypothesis 1); and (ii) inhibition of stimulation-evoked neurotransmitter release (Hypothesis 2).

On setting the predictions arising from this study against empirical observations, the most plausible hypothesis to emerge is that heptanol inhibits stimulation-evoked neurotransmitter release from the innervation of the vas deferens. Such a mode of action has not hitherto been proposed for heptanol, and we discuss its biophysical implications.

II. THEORY AND METHODS

Following Bennett & Gibson [4], the smooth muscle syncytium is modelled as a discrete 3-D grid, each node of which represents the internal medium of a muscle cell. Cell-to-cell connections are modelled as resistive links between the nodes. A second grid represents the adjacent interstitial region. The two grids are connected at every pair of corresponding nodes by an RC circuit representing the membrane of a muscle cell. For the activation of cells by neurotransmitter released from varicosities, membrane
representation incorporates current injection, modelled in terms of a driving potential \( E_v \), and a series conductance change \( g(T) = g_0 \alpha \exp(1-\alpha T/T_m) \). Here \( g_0 \) is the maximum membrane conductance and \( T \) is time normalized with respect to membrane time constant \( (T^* = \tau_m) \); \( \tau_m \) is given by the product of membrane resistance \( R_m \) and capacitance \( C_m \); \( \alpha \) is a "forcing function" dictating conductance time course, given by \( \alpha = \tau_m/T_p \), where \( T_p \) is the time to peak of \( g(T) \) [5].

The change in membrane potential at any node \( ijk \) \( (V_{ijk}) \) following injection of current into the syncytium is given [4] by the solution of:

\[
\frac{dv_{ijk}}{dt} + V_{ijk} = \lambda^2 (V_{ijk} + V_{i-j,k} - 2V_{ijk}) + \lambda^2 (V_{i,j,k} + V_{i-j,k} - 2V_{ij}) + \lambda^2 (V_{i,j,k} + V_{i,j-k} - 2V_{ij}) - R_m (V_{ijk} - E_v) g(T) \delta_{ijk}(\delta y) \ldots (1)
\]

where \( \Lambda = \sqrt{R_m/(R_i+R_o)} \), \( R_i \) being the resistance between adjacent intracellular nodes while \( R_o \) is the interstitial resistance between the adjacent extracellular nodes. Here \( \delta = 1 \) if \( ijk = \text{pq}r \), and denotes an active node, else \( \delta = 0 \) for passive nodes.

The set of differential equations resulting from application of (1) to all nodes in the syncytium was solved numerically to obtain solution for \( V_{ijk} \) with respect to time. The boundary conditions used were those appropriate for an insulated surface of the syncytium [4]. Simulations were performed using Fortran 77 code on a DEC ALPHA server running under OSF/l (UNIX). A double precision ordinary differential equation solver implementing the implicit Adams method of inputs, other parameters in equation (1) are taken from 141, and their properties of smooth muscle innervation by autonomic nerves, \( g_0 \), = 30 \( \mu \)S, \( \alpha \) = 14.3, (ii) loose-contact varicosity \( (LCV) \) input, using \( g_{LCV} = 1 \) \( \mu \)S, \( \alpha \) = 4.5. These parameter values and those for \( \tau_m \) and \( \tau_s \) for passive nodes.

**III. RESULTS**

**Hypothesis 1:** Cell-to-cell uncoupling in smooth muscle by heptanol.

The condition of cell-to-cell uncoupling in smooth muscle was simulated by increasing intercellular resistance \( R_i \) uniformly in the syncytium by factors of 10, 100 and 1000. This resulted in the following general effects.

eEJPs in passive cells declined partially in peak amplitude (by about 20%), accompanied by a noticeable increase in rise time \( (t_r) \) (Fig. 1A). In active cells, eEJP amplitudes increased in amplitude, with marked changes in \( t_r \) and time constant of decay \( (\tau_{decay}) \) (Fig. 1B). No qEJP-like events were obtained in active cells.

sEJP peak amplitudes progressively increased as \( R_i \) increased, accompanied by a flattening of the peak and increase of \( \tau_{decay} \) (Fig. 1C). In Fig. 1D we show amplitude histograms of sEJPs derived from the normal syncytium (Fig. 1D1) and at 1000Ri (Fig. 1D2). Note, at 1000Ri, (i) the increased clustering of observations in the lowest-amplitude bins; (ii) the emergence of very large sEJPs (peak amplitude 30-50 mV, which would occur at node 0). In contrast, the amplitude histogram of experimentally recorded sEJPs in the presence of heptanol does not change (not shown).

**Hypothesis 2:** Electrically unaltered smooth muscle syncytium, evoked neurotransmitter release inhibited.

This effect was simulated by reducing the spatial density of active CCVs and LCVs. The density of activation was reduced (without changing \( g_0 \)) to 1 in 2197 cells receiving CCV input and 1 in 8 receiving LCV input. In passive cells, the eEJP was strongly suppressed, by ~70-80% without change of time course (Fig. 2B). Simultaneously, in active cells, rapid evoked depolarizations, resembling sEJPs in time course, occurred at a short latency, thus generating qEJP-like signals (Fig. 2B). This pattern of changes is strikingly similar to that which emerges following heptanol action in active cells which exhibit qEJPs in experimental recordings [3].

qEJPs ride on a background depolarization. By subtracting the background depolarization (generated in a
Fig. 1. Effects of increasing $R_i$ (Hypothesis 1) on eEJPs (A) and sEJPs (B). Normal cell-to-cell coupling resistance is indicated by 1 (= $R_i$). Extent of increase of $R_i$ over normal levels indicated as follows: 2 = 10 $R_i$, 3 = 100 $R_i$, 4 = 1000 $R_i$. 

A: eEJPs

Passive cell showing effects of increase of $R_i$ on eEJPs at larger $R_i$'s. No change in amplitude of eEJPs. 

B: sEJPs

Active cell showing effects of increase of $R_i$ on sEJPs at larger $R_i$'s. 

V. DISCUSSION

Three salient features of the electrophysiology of the vas deferens in the presence of heptanol require to be explained consistently for any proposed mechanism of action of heptanol to be accepted, namely: i) decline of eEJPs in passive cells without change in time course; ii) emergence of qEJPs after the suppression of background depolarization in active cells; iii) persistence of sEJPs without alteration.

The commonly accepted hypothesis of heptanol action is that it uncouples cells from one another [1]. Our findings on simulated junction potential behaviour under conditions of increased $R_i$ (mimicking various degrees of probabilistic "block" of gap junction channels) suggest strongly that this mechanism of action cannot account for the experimental observations with 2.0 mM heptanol. Superficially the decline of eEJP amplitudes at passive nodes on increasing $R_i$ resembles the suppression of eEJP s by heptanol. However at a level of increase of $R_i$ that produces only ~20% suppression of eEJPs at passive nodes, the following effects are also observed: (i) eEJPs change in time course, especially in their rising phases (Fig. 1A); (ii) sEJPs are profoundly altered, in configuration as well as amplitude distribution (Fig. 1B). A combination of effects such as this was not observed experimentally. Furthermore the appearance of qEJPs could not be simulated by increasing $R_i$. On account of these discrepancies, it is difficult to accept that heptanol uncouples individual smooth muscle cells from one another to produce its experimentally observed effects.

In contrast, the alternative hypothesis, i.e. that of inhibition of evoked neurotransmitter release by heptanol, succeeded in predicting experimental observations adequately including (i) suppression of eEJPs in passive cells without...
change of time course, and (ii) emergence of distinct sEJP-like qEJPs in active cells (Fig. 2). If heptanol is found to have such an effect experimentally, this would have a number of significant implications. First, the effects of heptanol observed in some previous studies might require re-evaluation, since their explanation has rested on the inhibition of cell-to-cell coupling [1]. Second, it will be interesting to identify the precise target of heptanol action. In the process of evoked transmitter release, or "stimulus-secretion coupling" in the autonomic varicosity, several steps are interposed, including (i) Ca\(^{2+}\) influx linked to action potential invasion; (ii) interaction of Ca\(^{2+}\) with secretory vesicles; (iii) migration of vesicles to the varicosity surface membrane and their docking; (iv) fusion and poration of vesicle and surface membranes, leading to exocytosis of transmitter [9]. Very few agents are known that interfere specifically with any of these processes, and the action of heptanol would offer valuable insight into the mechanisms of evoked transmitter release. However, a necessary proviso is that the locus of heptanol action should be consistent with its lipophilic nature, and its proposed partitioning into the membrane lipid compartments of cells [1].

ACKNOWLEDGMENTS

We thank the All-India Council for Technical Education, Government of India, for financial support under the CAPYTI scheme of which RM is a beneficiary.

REFERENCES


