

TOPICAL REVIEW

Cellular electrophysiological principles that modulate secretion from synovial fibroblasts

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Abstract Rheumatoid arthritis (RA) is a progressive disease that affects both pediatric and adult populations. The cellular basis for RA has been investigated extensively using animal models, human tissues and isolated cells in culture. However, many aspects of its aetiology and molecular mechanisms remain unknown. Some of the electrophysiological principles that regulate secretion of essential lubricants (hyaluronan and lubricin) and cytokines from synovial fibroblasts have been identified. Data sets describing the main types of ion channels that are expressed in human synovial fibroblast preparations have begun to provide important new insights into the interplay among: (i) ion fluxes, (ii) Ca²⁺ release from the endoplasmic reticulum, (iii) intercellular coupling, and (iv) both transient and longer duration changes in synovial fibroblast membrane potential. A combination of this information, knowledge of similar patterns of responses in cells that regulate the immune system, and the availability of adult human synovial fibroblasts are likely to provide new pathophysiological insights.

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Background

It is well known that effective physiological function of human articular joints requires secretion of key lubricant substances. When this process fails, the likelihood of progressive osteoarthritis and/or rheumatoid arthritis

increases. In human articular joints, the synovial fibroblast cell population (often denoted FLS, from ‘fibroblast-like synovial cells’) plays a key role in this secretion. For example, both hyaluronan and lubricin are synthesized and secreted from FLS cells (Hui *et al.* 2012). Accordingly

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within the last decade, FLS preparations (*in vitro* and *in vivo*) have been used in multidisciplinary investigations of the fundamental mechanisms for the initiation and progression of rheumatoid arthritis (Huber *et al.* 2006; Lefèvre *et al.* 2009; McInnes & Schett, 2011; Bottini & Firestein, 2012; Juarez *et al.* 2012).

A comprehensive understanding of depolarization-induced secretion requires detailed insight(s) into the membrane-delimited electrophysiological processes that serve as ‘triggers’, as well as knowledge of intracellular signalling cascades in FLS preparations. In the case of the human synovial fibroblasts (sometimes also referred to as synoviocytes), a number of key underlying electrophysiological events have been identified (Bartok & Firestein, 2010). In addition, consistent patterns of responses of these cells to ligands and paracrine substances that are released into the synovial fluid during injury/inflammation and, or in the setting of progressive rheumatoid arthritis have been described (McInnes & Schett, 2011; Bottini & Firestein, 2012; Fleischmann, 2012; Hui *et al.* 2012).

The main purpose of this Topical Review is to summarize and integrate the electrophysiological data that are now available for mammalian synovial fibroblasts based mainly on results obtained from human FLS preparations maintained in short term culture. These findings provide some important new mechanistic information concerning electrophysiologically mediated secretion in FLS and related insights into disease aetiology and progression. Some of the important knowledge gaps that remain are also presented.

Baseline electrophysiology

Both conventional microelectrode techniques and patch clamp methods have been used in previous studies of the baseline electrophysiological characteristics and biophysical parameters of a number of different mammalian FLS preparations, including those from mouse, rabbit, bovine and human sources (Kolomytkin *et al.* 1997; Zimmermann *et al.* 2001; Large *et al.* 2010; Friebe *et al.* 2014). The published data, obtained predominantly from short term (1–3 days after enzymatic isolation) studies of low density cell cultures, yield single cell capacitance measurements in the range of 20–30 pF and corresponding input resistances between 0.5 and 2.0 GΩ (see Fig. 1). Measurements of the resting membrane potential of these types of isolated fibroblasts are surprisingly variable, ranging from –30 to –60 mV, with approx. –40 mV being most common (cf. Chilton *et al.* 2005 and Fig. 1).

There appear to be two main interrelated factors that contribute to this ‘scatter’ in the reported values of resting membrane potential. First, in relatively small cells, such as mammalian synovial fibroblasts, leakage

of the filling solution from the conventional microelectrode or patch clamp pipette can significantly change the intracellular milieu (Blatt & Slayman, 1983), and/or give rise to an appreciable electrochemical junction potential (Neher *et al.* 1992). Second, and perhaps more importantly, as shown in Fig. 1, patch clamp recordings from human synovial fibroblasts yield an N-shaped current–voltage relationship (I – V) when either rectangular or ramp ‘command voltages’ are applied to generate these biophysical descriptors. As a consequence, in the voltage range that is close to the apparent resting membrane potential, the synovial fibroblast input resistance is very large (5–15 GΩ). This results in a requirement for exceptionally high patch pipette to FLS surface membrane seal resistances, before the ‘full’ resting membrane potential value can be recorded consistently (Wilson *et al.* 2011). This consideration is particularly relevant when inwardly rectifying background K^+ currents are expressed. The effects of even very small changes in patch electrode-to-FLS ‘seal resistance’ are illustrated in Fig. 2. Note that when typical values for experimentally achieved seal resistance (for more detail see Wilson *et al.* 2011) are introduced into a mathematical model of a FLS cell, the apparent resting membrane potential changes because it is significantly shunted by the seal resistance, e.g. from –40 to 28 mV (cf. Bae *et al.* 2011). The curve fitting routines used to analyse these experimental data from individual human synovial fibroblasts and other mathematical modelling procedures are described briefly below.

Mathematical model. The current through the membrane of a synovial fibroblast, I_{Fib} , was defined as the sum of an inwardly rectifying current I_{Kir} , a large conductance Ca^{2+} -activated K^+ current I_{BK} , and an unspecific background current I_{b} :

$$I_{\text{Fib}} = I_{\text{Kir}} + I_{\text{BK}} + I_{\text{b}} \quad (1)$$

Each current was assumed to be instantaneous and dependent on membrane voltage, V_{m} . Parameters are listed in Table 1. A model to simulate the I – V relationship for I_{Kir} was developed previously by Iyer *et al.* (2004):

$$I_{\text{Kir}} = G_{\text{Kir}} \sqrt{[\text{K}^+]_{\text{o}}} \frac{1}{a_{\text{Kir}} + e^{\frac{b_{\text{Kir}}(V_{\text{m}} - E_{\text{K}})F}{RT}}} (V_{\text{m}} - E_{\text{K}}) \quad (2)$$

with conductance G_{Kir} , the K^+ reversal voltage E_{K} , and parameters a_{Kir} and b_{Kir} . E_{K} was determined by the Nernst equation. The formulation of the large conductance Ca^{2+} -activated K^+ current I_{BK} was based on Horrigan & Aldrich (2002):

$$I_{\text{BK}} = G_{\text{BK}} P_{\text{o}} (V_{\text{m}} - E_{\text{K}}) \quad (3)$$

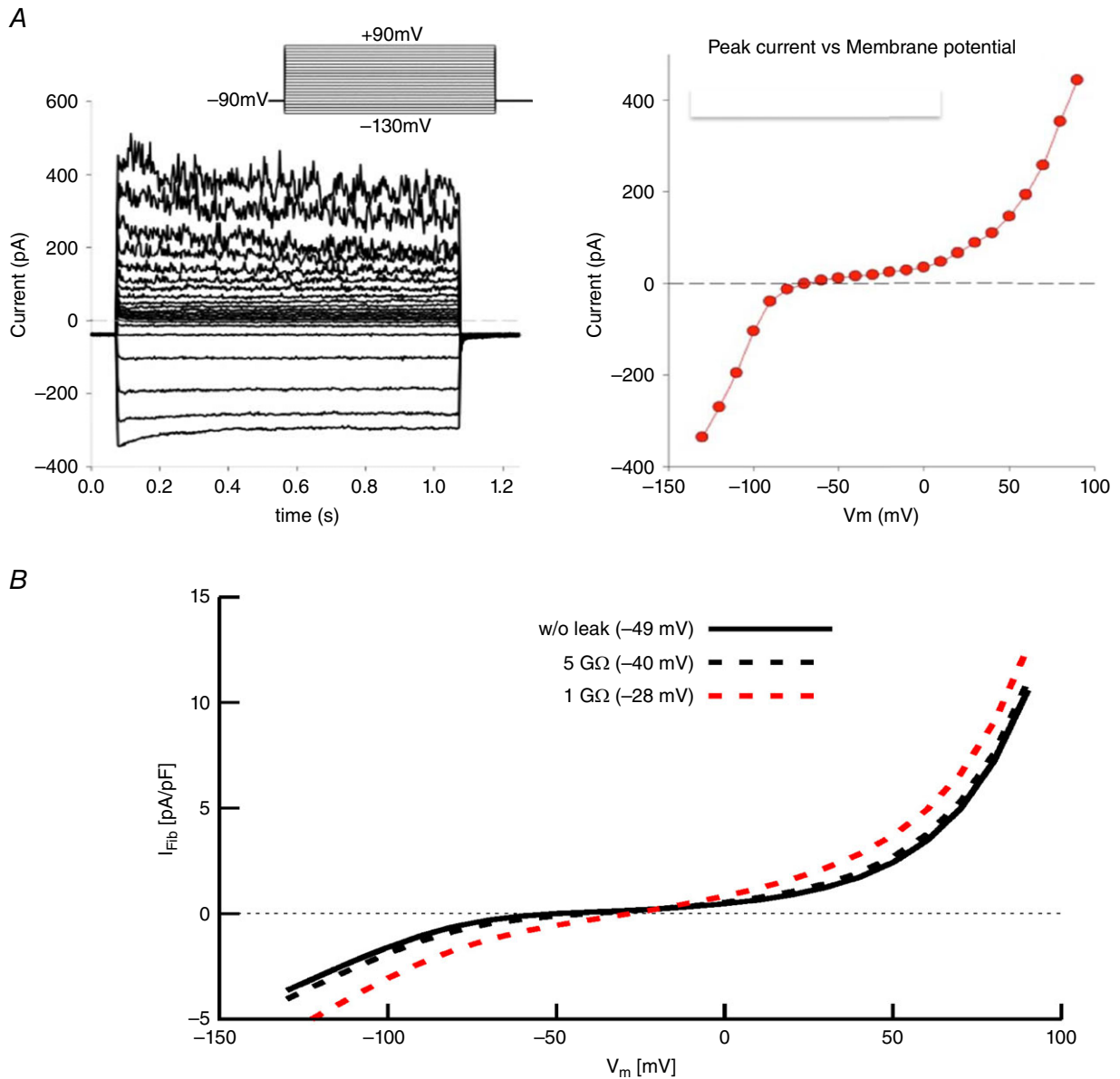


Figure 1. Current–voltage relations of a patch-clamped human synovial fibroblast

A, representative patch clamp recordings from a single human synovial fibroblast that had been in conventional 2-D cell culture for approximately 3 days. From an apparent resting membrane potential of approx. -40 mV, this cell was clamped to a holding potential of -90 mV and then 1 s rectangular voltage command steps were applied in 10 mV increments in the range of -140 to $+100$ mV. Distinct inward K^+ currents are activated by hyperpolarization; in addition, at least two different K^+ currents are activated positive to approx. 0 mV. The isochronal I – V relation on the right summarizes this information (W. R. Giles & R. B. Clark, unpublished observations). B, analyses of the effects of changes in patch microelectrode seal resistance on the apparent resting potential of a single enzymatically isolated human synovial fibroblast. Three simulated N-shaped I – V relationships are superimposed. The I – V relationship denoted by the black continuous line has been fitted to the experimental isochronal I – V data from 6 fibroblasts. Assuming that the patch electrode can form a ‘perfect’ seal, an apparent resting membrane potential of -49 mV is obtained. The data represented by dashed lines illustrate the changes in I – V curve shape and resting potentials when a linear leakage current (seal leakage) is added, assuming either a 1 GΩ (dashed red line) or 5 GΩ seal resistance (dashed black line) is introduced. As shown in the inset, the ‘seal leak’ depolarizes the ‘zero current membrane potential’ or apparent resting potential to approx. -40 mV (5 GΩ) or -28 mV (1 GΩ) from the value obtained with an infinite seal resistance -49 mV.

with the conductance G_{BK} and the open probability P_o . Under steady-state conditions P_o can be determined by:

$$P_o = \frac{L(1 + KC + JD + JKCDE)^4}{L(1 + KC + JD + JKCDE)^4 + (1 + J + K + JKE)^4}$$

$$L = L_0 e^{z_L e \frac{V_m}{kT}}$$

$$J = J_0 e^{-z_J e \frac{V_m}{kT}}$$

$$K = \frac{[Ca^{2+}]_i}{K_D} \quad (4)$$

using the parameters C , D , L_0 , z_L , J_0 , z_J and K_D . We also included a simple non-selective and time-independent background current I_b :

$$I_b = G_b(V_m - E_b) \quad (5)$$

having a conductance G_b and reversal potential E_b .

Several parameters in this model, i.e. G_{Kir} , G_{BK} , L_0 , z_L , J_0 , G_b and E_b , were identified using a stochastic optimization process developed previously by us (Abbruzzese *et al.* 2010). In short, we calculated a fit error between experimental and simulation data using

random perturbations of these parameters. In an iterative procedure, parameter sets with small fitting error were used to generate additional parameter sets, which were then evaluated again using the fitting error.

For simulations of the effect of seal leak currents we added a linear current:

$$I_{Leak} = G_{Leak}(V_m - E_{Leak}) \quad (6)$$

having a conductance G_{Leak} and a reversal potential E_{Leak} .

Very few studies aimed at determining the ionic basis for the resting potential of FLS preparations have been published. The available data suggest that in most of these isolated single cell preparations, time-independent or background Cl^- and K^+ currents both can contribute to the resting potential. Since it is now known (Ingram *et al.* 2008) that key physiological responses to environmental stimuli (including stretch) involve transmembrane influxes of Ca^{2+} and Na^+ , it is likely that a conventional Na^+/K^+ ATPase also contributes to the resting potential. Consideration of contributions from the Na^+/K^+ is important. This is

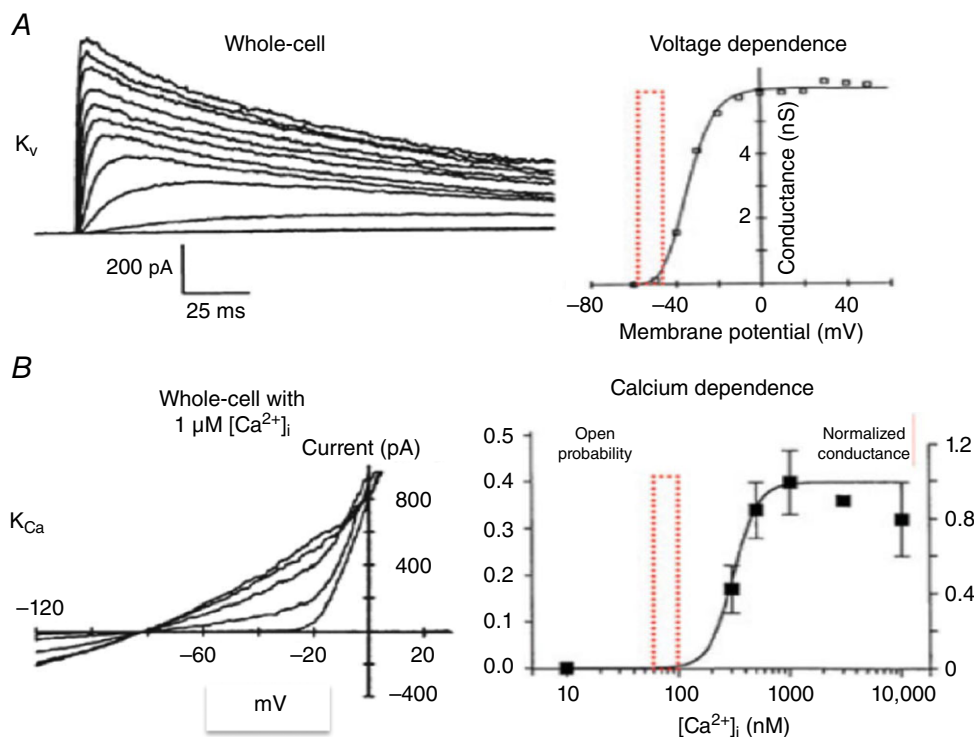


Figure 2. An illustration of plausible functional interactions of the K^+ currents expressed by synovial fibroblasts based on published biophysical data from T lymphocytes

A (left) shows a family of superimposed whole-cell K^+ currents generated in response to rectangular voltage clamp depolarizations. A (right) illustrates the voltage dependence of the activation of this 'delayed rectifier' K^+ current. The red box is included to highlight that the normal range of the 'resting potential' is near the foot of the activation curve in this preparation. B (left) consists of 5 superimposed I - V curves for the predominant Ca^{2+} -activated K^+ current in these T lymphocytes. B (right) illustrates Ca^{2+} dependence of channel opening for this Ca^{2+} -activated K^+ current. Here the red box shows the normal range of resting $[Ca^{2+}]_i$ values. These intermediate Ca^{2+} -dependent K^+ currents can increase significantly as $[Ca^{2+}]_i$ rises due to: (i) Ca^{2+} influx, (ii) Ca^{2+} release from the endoplasmic reticulum, or (iii) both (adapted from Cahalan & Chandy, 2009, with permission).

Table 1. Constants and parameters of fibroblast model

Name	Symbol	Value
Temperature (K)	T	294
Faraday's constant (C mol ⁻¹)	F	9.65×10^4
Gas constant (J K ⁻¹ mol ⁻¹)	R	8.31
Boltzmann constant (J K ⁻¹)	k	1.38×10^{-23}
Elementary charge (C)	e	1.60×10^{-19}
Intracellular K ⁺ concentration (mM)	$[K^+]_i$	120
Extracellular K ⁺ concentration (mM)	$[K^+]_o$	5
Intracellular Ca ²⁺ concentration (nM)	$[Ca^{2+}]_i$	129.92
Conductance for I_{Kir} (pA pF ⁻¹)	G_{Kir}	23.15
I_{Kir} parameter	a_{Kir}	0.94
I_{Kir} parameter	b_{Kir}	1.26
Conductance for I_{BK} (pA pF ⁻¹)	G_{BK}	1784.19
I_{BK} parameter	L_0	2.63×10^{-9}
I_{BK} parameter	Z_L	0.95
I_{BK} parameter	J_0	4237.36
I_{BK} parameter	Z_J	1.05
I_{BK} parameter (μM)	K_D	11
I_{BK} parameter	C	8
I_{BK} parameter	D	25
Conductance for I_b (pA pF ⁻¹)	G_b	10.56
Reversal voltage for I_b (mV)	E_b	-21.51

because since even a small outward electrogenic current generated by any of the known Na⁺/K⁺ pump isoforms would be expected to contribute outward currents that could hyperpolarize the cell some 2–10 mV. Since most published electrophysiological recordings have been made at room temperature (23°C), the contribution of small but significant electrogenic currents due to Na⁺/K⁺ pump turnover will be underestimated (Ingram *et al.* 2008; Wann *et al.* 2009; Large *et al.* 2010).

Both *in situ* and in some cell culture conditions, synovial fibroblasts are arranged in very close apposition with one another. In preparations isolated from the rabbit synovium, electrophysiological recordings (Kolomytkin *et al.* 1999) have identified significant cell-to-cell communication. This electrotonic current flow occurs through conventional mechanisms, i.e. connexin-based intercellular junctions. Interestingly, this group has also reported that one of the significant inflammatory mediators involved in rheumatoid arthritis, IL-7, can selectively modulate this intercellular communication. Specifically, application of IL-7 can result in reduction of this electrotonic communication, presumably as a result of inhibition of connexin-mediated intercellular current flow (Kolomytkin *et al.* 1997). We note also that a different cytokine, interleukin-1 β , has been shown to increase connexin-mediated cell-to-cell communication in a rabbit synovial fibroblast cell line that was maintained in tissue culture (Niger *et al.* 2010).

Primary cilia have been identified in mammalian FLS preparations (Rattner *et al.* 2010). By analogy

with other articular joint preparations (chondrocytes) these cilia are likely to be sensors for shear forces. They also appear to be preferential sites for initiating intracellular Ca²⁺ transients (Knight *et al.* 2009).

Triggers for depolarization–secretion coupling

The most comprehensive work concerning physiological ‘triggers’ and related intracellular signalling cascades that are essential for depolarization–secretion coupling from the synovial fibroblast has been published from the Levick and McHale laboratories (Momberger *et al.* 2006; Ingram *et al.* 2008; Wann *et al.* 2009; Large *et al.* 2010). Fundamental new insights were gained after this group developed an instrumented rabbit knee joint preparation. It provided the possibility of controlled repetitive displacements of this joint in conjunction with: (i) synovial fluid extraction and analysis, and (ii) selective changes in both the extra- and intracellular milieu of these synovial fibroblasts. Levick and colleagues demonstrated that the secretion of hyaluronan from rabbit synovial fibroblasts is strongly modulated by ‘joint activity’ (measured as the number of controlled displacements of the immobilized knee). This group (Momberger *et al.* 2006) also showed that this activity-dependent secretion was markedly reduced (perhaps even completely abolished) when $[Ca^{2+}]_o$ was removed from the superfusing medium/synovial fluid. In addition, when compounds that quite selectively block protein kinase C isoforms were added to the superfusate, much reduced secretion of hyaluronan was observed. Levick and his colleagues have also reported that mitogen-activated protein kinase activation is essential for this Ca²⁺-mediated ‘activity-dependent’ hyaluronan secretion and that this mechanosensitive hyaluronan secretion results from a Ca²⁺-dependent transcription/translation process (Wann *et al.* 2009). Patch clamp experiments done on a subset of synovial fibroblasts isolated from this rabbit knee joint preparation showed that a number of different K⁺ channels and L-type Ca²⁺ channels were expressed. Importantly K⁺ channel blockers depolarized the resting membrane potential, and a large increase (to 60 mM) in extracellular $[K^+]$ produced a significant rise in $[Ca^{2+}]_i$ (Large *et al.* 2010).

Environmental sensors in synovial fibroblasts

Ion channels that are expressed in the surface membrane of synovial fibroblasts react to a wide variety of ligands that are present in synovial fluid. One of the earliest reports of this (Christensen *et al.* 2005) showed that a decrease in extracellular pH (pH_o) resulted in a substantial increase in intracellular Ca²⁺, $[Ca^{2+}]_i$, in cultured synovial fibroblasts. This group also showed that this response

to an acidic pH_o is G-protein mediated, and requires a transmembrane Ca^{2+} influx as a 'trigger'. Additional work has established that this increase in $[\text{Ca}^{2+}]_i$ is initiated by heat-sensitive transient receptor potential (TRP) channel activation (cf. Vriens *et al.* 2004; Kochukov *et al.* 2006; Beech, 2013).

Subsequent papers have characterized this response to reduced pH_o in more detail. In human synovial fibroblasts (Kolker *et al.* 2010) a large reduction in pH_o (to approx. 5.5) consistently produced a substantial increase in $[\text{Ca}^{2+}]_i$ as well as an associated stimulation of hyaluronan secretion. Additional experiments directed toward identifying the primary pH sensor revealed the acid-sensitive Na^+ channel ASIC III (Immke *et al.* 2001) as one of the essential elements in this signal transduction pathway. Small interfering (si)RNA-mediated ASIC III knock-down manoeuvres (Kolker *et al.* 2010) eliminated the significant increase in $[\text{Ca}^{2+}]_i$ in response to pH_o reduction to 5.5. However, since ASIC III channels are permeable to both Na^+ and Ca^{2+} , it is not known whether the 'trigger cation influx' (Vriens *et al.* 2004; Itoh *et al.* 2009; Bartok & Firestein, 2010) is mainly Na^+ , Ca^{2+} or both under these conditions.

Somewhat similar studies by the Muraki group (Itoh *et al.* 2009) addressed the possibility that the initial cation influx that initiates this depolarization–secretion coupling cascade was mediated by activation of TRP channels. In their preparation, human synovial fibroblasts, TRPV4 channel activation by an acidic pH_o resulted in a marked increase in $[\text{Ca}^{2+}]_i$. Results from their TRP channel-specific knockdown experiments established that this response to pH_o had an obligatory dependence on TRPV4 expression. They also showed that depletion of the Ca^{2+} from the intracellular stores (the endoplasmic reticulum) markedly reduced the ability of pH_o to increase $[\text{Ca}^{2+}]_i$.

One of the most robust and translationally relevant data sets describing TRP channel activation in FLS preparations (Beech & Sukumar, 2007; Xu *et al.* 2008) showed that the endogenous redox agent thioredoxin (Holmgren, 1985; Yoshida *et al.* 1999; Jikimoto *et al.* 2001; Lemarchal *et al.* 2006, 2007; Huili & Wan, 2013) could potentially activate a TRP channel-mediated current. This pattern of responses was recorded using both heterologous expression systems and synoviocytes isolated from patients that had been diagnosed with rheumatoid arthritis. In this study, results from point mutation protocols revealed that specific cysteine residues in both TRPC5 and TRPC3 were the thioredoxin target(s).

Ligand-induced increases in $[\text{Ca}^{2+}]_i$ can represent an important first step in both evoked and constitutive cellular secretion cascades. In many types of endocrine cells, this increase in $[\text{Ca}^{2+}]_i$ can then activate distinct classes of Cl^- or K^+ channels and thereby cause significant and long-lasting changes in the membrane potential

of the target cells. For example, a recent paper by Friebe *et al.* (2014) demonstrated a functional role for one specific type of Ca^{2+} -activated K^+ channel in human synovial fibroblasts. This work, using primary cell cultures, identified the expression of the intermediate Ca^{2+} -activated K^+ conductance KCa3.1 or KCNN4 (Berkefeld *et al.* 2010; Hu *et al.* 2012; Bi *et al.* 2013) in these FLS preparations. It also showed that modulation of the expression level of KCa3.1, as judged by both patch clamp recordings and antibody experiments, significantly altered the proliferation rates of these cells. In addition, Friebe *et al.* (2014) reported that a well-known synovial fibroblast stimulant, transforming growth factor $\beta 1$ (TGF- $\beta 1$) (cf. Pohlers *et al.* 2007), can significantly increase transcription, translation and expression of this particular K^+ channel. In contrast, inhibition of this Ca^{2+} -activated K^+ current resulted in reduced (i) proliferation and (ii) secretion of synovial fibroblast-derived pro-inflammatory mediators IL-6, IL-8 and MCP-1. Since these synovial fibroblasts were obtained from donors that had a rheumatoid arthritis (RA) diagnosis, this K^+ current, KCa3.1, could be a target for discovery of anti-RA agents. This ion channel in T-cells has been selected as a primary target for drug development to alleviate immune and autoimmune disorders (Cahalan & Chandy, 2009).

Integration of ion channel data

It is known that mechanical perturbations or ligand-based stimulation of isolated synovial fibroblasts, either *in situ* or in cell culture settings, can consistently give rise to significant and relatively long lasting changes in $[\text{Ca}^{2+}]_i$. There is also evidence that these changes in $[\text{Ca}^{2+}]_i$ can function as one of the second messengers for activation for a number of linked signalling cascades that modulate both evoked and constitutive release of hyaluronan, cytokines and growth factors. These substances strongly modulate for synovial fibroblast (RA) and articular joint function, both under baseline conditions and in disease states. However, more detailed information is needed regarding important aspects of these $[\text{Ca}^{2+}]_i$ -mediated signalling pathways. The trigger or initial source of the Ca^{2+} that is the electrophysiological signal for endoplasmic reticulum-mediated intracellular Ca^{2+} release needs to be identified. Data obtained from mouse and human FLS preparations maintained in culture suggest that this initial Ca^{2+} influx arises from activation of TRP channels (Itoh *et al.* 2009) with the TRPV4/V1 and TRPC3/5 family being the strongest candidates (Kochukov *et al.* 2006; Engler *et al.* 2007). However, information is lacking concerning the voltage dependence of the Ca^{2+} entry pathway(s), and further insights into the cation selectivity, ligand sensitivity and pharmacological profile are needed. Moreover, in the well-studied rabbit

synoviocyte preparation (Large *et al.* 2010) L-type Ca^{2+} channel expression and transmembrane Ca^{2+} currents have been identified.

By analogy with the known sequence of events in mammalian endothelial cells, even a very small Ca^{2+} or Na^+ influx through e.g. TRPV4 channels (particularly if it is spatially localized to an immediate subplasma membrane space) can trigger a much larger Ca^{2+} -induced Ca^{2+} release from intracellular stores, the endoplasmic reticulum (Stathopoulos *et al.* 2012; Sonkusare *et al.* 2012; Dunn *et al.* 2013). The resulting changes in Ca^{2+} can serve both the 'AM and FM signalling modalities' for Ca^{2+} in these FLS preparations as happens in many other cell types (Berridge *et al.* 2000; Farley & Sampath, 2011). Importantly, some aspects of the maintained or plastic signalling that is initiated by an increase in $[\text{Ca}^{2+}]_i$ may not require maintained increases in $[\text{Ca}^{2+}]_i$.

Many important aspects of the Ca^{2+} signalling 'tool kit' in synovial fibroblasts remain to be elucidated or further defined (cf. Berridge *et al.* 2000). It is clear, however, that the initial transmembrane trigger signal (Ca^{2+} influx) is very small. However, it gives rise to a much larger Ca^{2+} release from the endoplasmic reticulum. As in other non-excitabile cells, the spatial localization of the Ca^{2+} entry 'pathways' on the plasma membrane offer possibilities for signal integration and amplification. Much of the Ca^{2+} that enters FLS cells is rapidly bound to intracellular buffers. This process can 'shape' the Ca^{2+} transient and also regulate (limit) its spatial profile. Calmodulin functions both as a rapid Ca^{2+} buffer and as a Ca^{2+} sensor. It can strongly activate downstream effectors and thus can result in changes in, e.g., ion channel function, metabolism or gene transcription. Some of these integrative processes are mediated by Ca^{2+} /calmodulin-dependent protein kinase II and/or protein kinase C isoforms. Often the end result of a brief Ca^{2+} transient is a maintained cellular response. A well-documented example of this is the Ca^{2+} influx plasticity in the mammalian brain (Ma *et al.* 2015). In the hippocampus (and elsewhere) details of the frequency dependence or repetitive nature of the changes in $[\text{Ca}^{2+}]_i$ signalling are known to have differential effects on distinct pools of transcription factors, e.g. nuclear factor of activated T-cells (NFAT) or intercellular latent gene regulatory proteins (NF κ B) (Berridge *et al.* 2000).

In the case of FLS preparations, plausible candidates for the initial repetitive stimulus include: (i) cyclic changes in surface forces that are transduced by stretch-activated channels, (ii) shear forces, and/or (iii) pulsatile release of ATP from other FLS cells, nerve endings, or emigrated neutrophils as a consequence of inflammation.

Increases in $[\text{Ca}^{2+}]_i$ can also activate one or more Ca^{2+} -sensitive ion channels that are localized in the plasma membrane. A prominent feature of most recordings from the synovial fibroblast is the activation of BK or

large conductance Ca^{2+} -activated K^+ channels. As shown in Fig. 1, I_{KCa} can give rise to a characteristic very 'noisy' outward current. It is activated at very positive (depolarized) membrane potentials and therefore is not of physiological significance. However, the hallmark feature of activation of these BK channels is a shift of their activation to more hyperpolarized membrane potentials as $[\text{Ca}^{2+}]_i$ increases (Berkefeld *et al.* 2010; Hill *et al.* 2010). Moreover, this subset of Ca^{2+} -activated K^+ channels has been shown to be sensitive to carbon monoxide (CO), hydrogen sulfide (H_2S), and a number of other physiological substances of interest within the framework of articular joint physiology (cf. Orio *et al.* 2002).

It is also apparent that a second and distinct Ca^{2+} -dependent K^+ channel subset, KCa3.1 , can be activated by the large increases in $[\text{Ca}^{2+}]_i$ that are generated by, e.g., increases in ATP and/or acidification of pH_o . Both of these take place in the setting of rheumatoid arthritis. This so-called intermediate Ca^{2+} -activated K^+ current exhibits completely different biophysical properties, pharmacology characteristics and cellular electrophysiological features from those for BK current (Gao *et al.* 2010; Bi *et al.* 2013; Friebel *et al.* 2014). Perhaps the most important difference is that these KCa3.1 channels can be strongly activated in the physiological range of membrane potentials. In the synovial fibroblast, and in immune cells (Cahalan & Chandy, 2009), these KCa3.1 channels can be blocked on their distinct pharmacological profile. In T lymphocytes this KCa3.1 current is a primary target for intrinsic substances and new synthetic agents (pro-drugs), some of which continue to be evaluated with promising results in preclinical or clinical settings (Wulff *et al.* 2000).

Some important information regarding the relationships among: (i) cellular electrophysiology, (ii) some aspects of $[\text{Ca}^{2+}]_i$ -mediated second messenger signalling pathways, and (iii) hyaluronan secretion in mammalian synovial fibroblasts is now available (Wann *et al.* 2009). However, additional work is needed before feasible drug-sensitive targets that can be used for manipulating the function of synovial fibroblasts can be identified. Similarly, although some overall processes that regulate depolarization–secretion coupling are known, very important aspects of physiological and pharmacological regulation remain to be identified. One example is the requirement to understand more completely the interrelated effects of selective or collective (double hit) activation of the populations of distinct K^+ channels that are expressed in these synovial fibroblasts. As has been shown in the T lymphocyte, and is illustrated in Fig. 2B, when the intermediate conductance Ca^{2+} -activated K^+ ($I_{\text{KCa3.1}}$) is turned on, the target cell (T lymphocyte or FLS cell) hyperpolarizes markedly. This hyperpolarization can markedly augment Ca^{2+} entry through TRP channels or through other Ca^{2+} -selective

pathways (cf. Cahalan & Chandy, 2009; cf. Funabashi *et al.* 2010).

Summary

Cell physiology/biophysics principles. Preliminary electrophysiological results from our laboratories (T.A.S., W.R.G.) indicate that at baseline (in the absence of paracrine, mechanical or autonomic nervous system stimulation), the synovial fibroblast typically exhibits an N-shaped resting or background $I-V$ relationship. The two most prominent transmembrane ionic currents that are responsible for this are: a time-independent inwardly rectifying background K^+ current that is activated at hyperpolarized membrane potentials (i.e. negative to approx. -40 mV), and a Ca^{2+} -activated K^+ current (the large conductance, BK_{Ca}) that is activated at membrane potentials positive to approx. -10 mV. This N-shaped background $I-V$ has a number of functional consequences, including:

- The prominent 'flat region' of this type of $I-V$ curve results in significant technical challenges that limit accurate and/or reproducible measurement of membrane resting potential, E_m (Wilson *et al.* 2011).
- Since the levels of expression of both of these K^+ currents are quite variable, these FLS cells (when studied individually), would be expected to show considerable 'scatter' in their E_m values (see Large *et al.* 2010, Fig. 3).
- Agonists or paracrine factors that 'activate' ligand-gated currents when E_m is in the high resistance (flat) region of the $I-V$ curve, will have very significant effects on E_m . This is because even quite small changes in E_m can strongly modulate other essential cellular functions, such as resting $[Ca^{2+}]_i$ levels and/or intercellular coupling.
- The expression of inwardly rectifying K^+ current not only regulates E_m but can also modulate intercellular coupling. Accordingly, cell-to-cell communication in the FLS syncytium may be sensitive to very small changes in $[K^+]_o$ (cf. Jantzi *et al.* 2006; Smith *et al.* 2008; Anumonwo & Lopatin, 2010).
- The effectiveness of compounds that activate most TRP channel isoforms would be expected to show a strong dependence on E_m . The combination of the effects of shape of this N-shaped 'background' $I-V$ relationship and the fact that the reversal potential for many TRP channels is approximately -20 mV (i.e. within the flat region of the baseline $I-V$) combine to result in this sensitivity.
- Finally, this N-shaped $I-V$ curve makes it likely that even very small intrinsic current sources, e.g. the electrogenic current due to the Na^+/K^+ pump, can

alter E_m . Accordingly, cell metabolism may be linked to the electrophysiological phenotype (cf. Jacquemet & Henriquez, 2008; Clark *et al.* 2010, 2011; Wilson *et al.* 2011) of FLS preparations.

Translational significance. Knowledge of the most significant electrophysiological principles that regulate the synovial fibroblast may provide novel insights into progressive rheumatoid arthritis (Imamura *et al.* 1998; cf. McInnes & Schett, 2011). It may also assist in identification of primary or 'double hit' drug development efforts (Oshima *et al.* 2000; Kunisch *et al.* 2007; Zhao *et al.* 2008; Petroff *et al.* 2012) and contribute to rational decisions for pain management either following acute injury or in settings involving progressive deterioration of the articular joint.

By analogy with the work of Cahalan & Chandy (2009) on human T-cells, the intermediate conductance Ca^{2+} -sensitive K^+ channel $KCa3.1$ may be a viable drug target in the synovial fibroblast (cf. Friebel *et al.* 2014). It is also worth recalling that ion channel trafficking to the surface membrane may be altered in disease settings. In addition, it is now well known that a number of different ion channel α subunits, including those for $KCa3.1$, can strongly influence their parent cell phenotype and function (Friebel *et al.* 2014). Importantly, these effects can be significant even when these channel subunits are in their silent or non-conducting states (Kazcmarek *et al.* 2006).

It is also worth noting that regulated secretion of other articular joint lubricants such as PGR4 or lubricin (Nugent-Derfus *et al.* 2007) may be able to be enhanced by using electrophysiological insights/approaches. Hyperpolarizing FLS cells prior to their activation with, e.g., natural substances (ATP) (cf. Millward-Sadler *et al.* 2004; Romanov *et al.* 2008), synthetic steroids (Ciurtin *et al.* 2010), or mechanical 'oscillation' may activate or enhance secretion of essential lubricant molecules, cytokines and/or ATP (Hu *et al.* 2012).

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Additional information

Competing interests

None declared.

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