MODELING THE ELECTRICAL ACTIVITY OF A UTERINE CELL A MATHEMATICAL MODEL APPROACH

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Abstract: Preventing preterm labour consists nowadays in a major field in obstetrics researches. It seems important to understand different physiological mechanisms responsible for myometrial contractions at term. Therefore our aim in this paper is to present a first approach of a mathematical model simulating the electrical uterine activity. This model is based on the basic electrophysiological model, Huxley and Hodgkin model, and on biophysical parameters obtained by patch clamp experiments found in the literature.

Introduction

Preterm labour consists nowadays in a major obstetrical problem. Preterm child suffer severe neurological problems. Uterine contractions, when rhythmic and coordinated, lead to the expulsion of the foetus at delivery. The principal component of the uterus responsible of these contractions is the uterine muscle (myometrium).

It seems important to better understand the causes of preterm labour and the different physiological mechanisms responsible for myometrial contraction leading to the expulsion of the foetus.

Since electrophysiological modelling is an important tool to a better understanding of physiological and biological systems, our aim in this paper is to develop a mathematical model of myometrial electrical activity; this electrical activity is related to myometrium action potentials.

The base mathematical core of electrophysiological models is the Huxley and Hodgkin (HH) model developed on 1952 [1]. Based on it, several mathematical models have been implemented that mainly simulate the electrical cardiac activity [2], [3] and [4]. While a variety of electrophysiological models describing bursting electrical activity have been recently developed (pancreatic [5], neuronal [6]) it seems that there is no mathematical model of the uterine electrical activity.

Since the electrical activity of a uterine cell, described by a resting potential, a slow wave, and a fast wave (action potentials) is generated due to the flow of ions through the cellular membrane, we present in this review, a model describing the activation and inactivation kinetics of the main ionic channels primarily involved in myometrial contraction. A system of differential equations based on Huxley and Hodgkin model [1] has been developed. This system simulates a train of action potentials observed in a uterine cell at term.

Materials and Methods

HH consider the total membrane current divided in two components: a capacitive current I_c and an ionic current I_{ion}. The latest is associated to the flow of ions through membrane ionic channels.

The electrical properties of a uterine cell can be described by a differential equation (1):

$$I_{ion} + C_m dV_m / dt = I_{stim} \quad (1)$$

I_{stim} being the stimulation current (external applied current).

The ionic current I_{ion} showed in equation (2) is divided into inward and outward currents. Inward current is composed primarily by sodium I_{Na} and calcium I_{Ca} currents. The main outward current is the potassium one, I_K. We distinguish potassium voltage dependent current $I_{K(V)}$ and a potassium calcium dependent current $I_{K(Ca)}$.

I_L is the leakage current, the remaining current, primarily consisting in chloride ions.

$$I_{ion} = I_{Na} + I_{Ca} + I_{K(V)} + I_{K(Ca)} + I_L \quad (2)$$

Each ionic current, I_i, is assumed to be related to the membrane voltage, V_m, based on voltage dependent gating properties, equation (3).

$$I_i = g_i(V - E_i) = G_i m_i^x h_i^y (V - E_i) \quad (3)$$

E_i is the equilibrium potential of the ion i. This equilibrium potential is created by the unequal ion distribution between intra and extra cellular space and its expressed by the Nernst formula, equation (4) :

$$E_x = RT \ln\left([X]_o / [X]_i\right) / zF \quad (4)$$

Ionic concentrations in the intra and extra cellular space are assumed to be constant during an action potential, except for calcium intracellular concentration, $[Ca^{2+}]_i$. These intra and extra cellular concentrations are listed in table 1:

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Table1: Intra and extra cellular concentrations [7],[8]

Ion X	$[X]_i$(mM)	$[X]_o$(mM)	E_i(mM)
Na^+	25	140	45.7
Ca^{2+}	0.0015	1.5	122
K^+	150	5	-85
Cl^-	60	135	-21.5

G_i is the maximum ionic conductance when all the channels are open. But ionic channels can be either in an open, partially open or closed state. The probability that a channel is open is $m_i^x h_i^y$, where m and h are so called gating variables, which are voltage dependent variable. There is x activating m gates and y inactivating h gates.
The voltage gating dynamics of these gating variables are expressed as follow, equation (5):

$$dm/dt = (m_\infty - m)/\tau_m \quad (5)$$

Where m_∞ is the steady state activating gate variable, τ_m is the associated time constant needed to reach the steady state from an initial value. A similar equation describes the gating variable h.

By using these equations, each myometrial ionic current can be expressed by a voltage dependent equation, equation (3). Generally, the data used in electrophysiological models to define the gating of each ionic current for each individual mathematical ion models are provided by voltage clamp experimentations. In our model, data are based on patch clamp experiments found in the literature.

Main Ionic expressions

Two types of inward current have been described for pregnant rat [9], [10] and for pregnant woman [11] cells at the end of term. There are sodium and calcium current.

Sodium current, I_{Na}: It is the largest inward current, which is quickly activated and inactivated. The activation kinetic fits with a fourth power function; equation (6), with voltage dependent τ's varying between 0.39 ms at -20 mV and 0.18 ms at 20 mV [9].

$$I_{Na} = I_\infty (1 - \exp(-t/\tau))^4 \quad (6)$$

The steady state voltage activation relation follows Boltzman distribution, equation (7), with half activation occurring at -21 mV and a slope factor of 5 mV [9].

$$m_{Na\infty} = 1/[1 + \exp([V_m + 21)/-5]] \quad (7)$$

Inactivation of I_{Na} has been described as a single exponential, with also a small time constant that varies from 0.77 ms at -10 mV to 1.4 ms at 30 mV. The steady state voltage inactivation relation also follows a Boltzmann distribution with half inactivation occurring at -58 mV and a slope factor of 8.7 mV [9].

Since activation and inactivation time constants of I_{Na} are relatively small, the channel gating variable m_{Na} and h_{Na} remain very close to their steady state values during an action potential and can be set to their steady state value. Consequently, we can suggest the following equation (8) as expression of the sodium current:

$$I_{Na} = G_{Na} m_{Na\infty}^4 h_{Na\infty} (V_m - E_{Na}) \quad (8)$$

Calcium current, I_{Ca}: L-type calcium voltage dependent current has been considered as the primarily inward calcium current in rat uterine cell [12], [9]. In addition, both L-type and T-type calcium voltage dependent current have been observed on human myometrial cells [13], [14]. Since T-type Ca^{2+} current is inactivated at a potential of -60 mV, at resting potential -50 mV, a large fraction of the transient-type calcium channel is inactivated releasing the question of its physiologic role in the generation of action potential [13],[14]. L-type Ca^{2+} channels therefore contribute primarily to the depolarisation of the membrane and to the release of the bursting electrical activity. It was found that these voltage dependent channels are likely to be inactivated by variation of calcium intracellular concentration $[Ca^{2+}]_i$ [15]. It was likely found that the majority of calcium transport takes place through human uterine L-type channels during labour [14]. Consequently, our work will focus primarily on calcium L-type current.

Activation of I_{Ca} have been described by [9] by using a square function, with a relatively small voltage dependent time. Therefore, we suppose that the gating activation variable, m_{Ca}, is equal to the steady state activation gating variable, $m_{Ca\infty}$. The steady state voltage-activation relation follows Boltzman distribution, where half activation occurres at -8 mV and slope factor is 6.6 mV.

Calcium inactivation is more complex than sodium inactivation. Young et al. [14], have found a great deal of cell-to-cell variability of the decay time course of the L-type current. Most of the cells have been found expressing an inactivation time on the order of tens of milliseconds. These data concerning the inactivation times of calcium woman currents I_{Ca}, match the results found by Yoshino and al for pregnant rat uterine cells [9]. In this case, inactivation time has been described by two exponential phases. The first one, voltage dependant, is the faster one, showing a U shaped relation explained by calcium effect inactivation. The second one is the slower one but is associated with too scattered data. The steady state voltage inactivation relation also follows a Boltzmann distribution described by [9] with half inactivation=-34 mV voltage and a slope factor=5.4mV.

We propose to describe the calcium current expression as follow, equation (9):

$$I_{Ca} = G_{Ca} m_{Ca\infty}^2 h_{Ca} f_{Ca} (V_m - E_{Ca}) \quad (9)$$

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Where h_{Ca} is the voltage dependent inactivation variable described by the first order differential equation, and f_{Ca} describes the inactivation by calcium mechanisms. Since myometrial calcium current have been found similarly to the myocardial one [14], we proposed an f_{Ca} expression, equation (10), inspired from the one described for myocardial cell by [4], [16], where $K_{1/2}$ is the half activation concentration for Ca^{2+}.

$$f_{Ca} = 1/[1 + [Ca^{2+}]_i / K_{1/2}{}^2] \quad (10)$$

Calcium plays an important role in myometrial contraction. The increase in calcium intracellular concentration may be considered as the first factor of membrane depolarisation leading to action potential. We thus propose to use the following expression, equation (11), to describe the variation of $[Ca^{2+}]_i$

$$d[Ca^{2+}]_i / dt = f(-\alpha I_{Ca} - K_{Ca}[Ca^{2+}]_i) \quad (11)$$

We have to identify a combination of these parameters permitting to obtain a train of action potentials.

Potassium current, I_K: The involvement of K^+ voltage and Ca^{2+} dependent channels in the quiescence of the uterus during pregnancy and for the initiation of contractions in labour is not fully understood [17]. These K^+ channels play an important role in terminating an action potential and for membrane cellular repolarisation [18], preventing thus long tetanus contractions leading to foetal death [17]; [19]; [20]; [21].

As cited above two major types of potassium currents have been identified in uterine cell, $I_{K(V)}$ and $I_{K(Ca)}$ currents.

Potassium voltage dependent current, $I_{K(V)}$: Three populations of K^+ voltage dependent current have been described by [19] : two inactivating components (I_{K1}, 67% and I_{K2}, leading to 23% of the total $I_K(V)$) and the third I_{K3} which is non inactivating and represent the 10% of the total I_K. The two inactivating components I_{K1} and I_{K2} observed by Wang in rat myometrium have also been observed by Knock [17] in pregnant human.

Consequently, $I_{k(V)}$ can be expressed by the sum of the three voltage dependent component, equation (12).

$$I_k = I_{k1} + I_{k2} + I_{k3} \quad (12)$$

As delayed rectifier current, we suggest for each potassium component current one activating gate, equation (13), which dynamics is described by the first order differential, equation (5). We also suggest that the steady state voltage dependent is also expressed by a Boltzmann distribution with a slope factor, S and a half activation potential, $V_{1/2}$.

$$\begin{aligned} I_{K1} &= G_{K1} n_{K1} h_{K1} (V - E_K) \\ I_{K2} &= G_{K2} n_{K2} h_{K2} (V - E_{K2}) \\ I_{K3} &= G_{K3} n_{nK3} (V - E_{K3)} \end{aligned} \quad (13)$$

Potassium calcium dependent current, $I_{K(Ca)}$: It has been shown that due to their high density per cell, their large conductance and their Ca^{2+} sensitivity, K_{Ca} channels may contribute to membrane repolarisation and to the maintain of the resting membrane potential in a uterine cells [22]. It has also been shown that P_0, ratio between the open time and the total time channel, fits the Hill function, equation (14)

$$P_0 = 1/[1 + (K_{1/2} / [Ca^{2+}]_i)^N] \quad (14)$$

Where $K_{1/2}$ is the half-activation concentration and N is the Hill coefficient. The Ca^{2+} concentration for half activation ($K_{1/2}$) of the channel was about 10µM at 40 mV and 20µM at -50 mV. The Hill coefficient N, remain constant equal to 2 for both potentials [22].

Based on the model proposed by Plant (1978), [23], authors generally use the followed I_{KCa} expression, equation (15).

$$I_{K(Ca)} = g_{K(Ca)}[Ca^{2+}]_i (V - E_k) \quad (15)$$

Where $g_{K(Ca)}[Ca^{2+}]_i$ is the fraction of open channels expressed in function of the total conductance $g_{K(Ca)}$ and of $[Ca^{2+}]_i$ where channels transit from open to closed [7]. We express the fraction of open channels $g_{K(Ca)[}Ca^{2+}]_i^N$ as a function of Nernst coefficient equal to 2, equation (16) :

$$g_{K(Ca)}[Ca^{2+}]_i^N = G_{K(Ca)} P_0 \quad (16)$$

Where $G_{K(Ca)}$ is the maximal conductance due to K(Ca) channels.

Results

We have tested the proposed uterine cell model by comparing its different components and the simulated obtained signals to conditions that emulate experimental results found in the literature.

We have first validated each ionic current expression separately. We have compared the single ionic expression predictions with corresponding measured data found in the literature and based on voltage clamp experiments. So, we assume to separately analyse each ionic channel by studying its variations and its time evolution function of a holding potential.

Sodium current: By testing the sodium current in function of different membrane potentials, we observe the following variation. Figure (1) presents activation at about -20 mV, a peak of 5.1µA/cm^2 at 0 mV, and a reversal potential at 85 mV. These results are in agreement with the available data described by [9].

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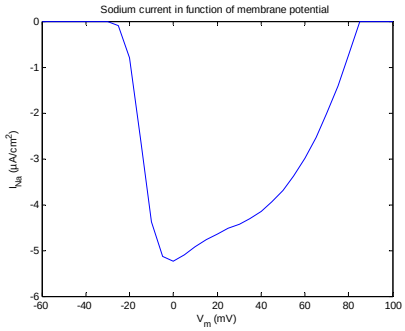

Figure 1 : Simulation of sodium current variation as function of membrane potential (the holding potential used in voltage clamp experiments).

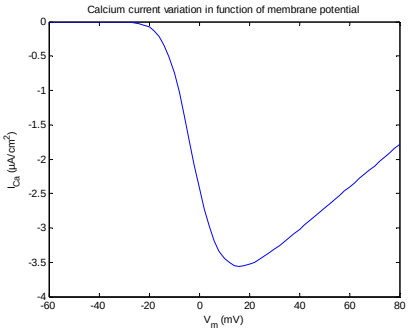

Figure 2 : Simulation of calcium current variation in function of membrane potential (the holding potential used in voltage clamp experiments).

Calcium current: Figure 2, presents the calcium current variation obtained in function of membrane potential. The calcium current is expressed with two activation gates, one inactivation voltage dependent gate and f_{Ca} inactivation by calcium mechanisms. The shape of the curve, (maximum at 3.5µA/cm^2) and its activation at about -30 mV, coincide with experimental voltage clamp data's found in the literature as in [9] or in [14].

Potassium current: The obtained potassium current also presents properties similar to the ones observed in the literature. The current-membrane potential curve is a linear relation with two different slopes for two different holding potentials (HP). Figure 3, shows that at HP=-50 mV, the outward current was about 50% of that at HP=-80 mV. These results match with the curves described by [19].

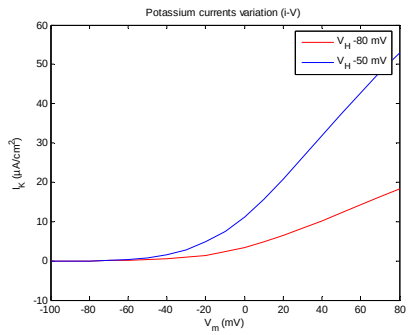

Figure 3 : I-V linear variation of K^+ currents for two holding potentials.

Model test: By combining the whole individual current expressions, our model contains nine differential equations describing fast inward sodium current, slow inward calcium current, potassium voltage dependent current and calcium dependent current. We consider initially a null leakage current. So, in response to a stimulation current of 1.5µA/cm^2, our model generates a train of action potentials presented in figure 4.

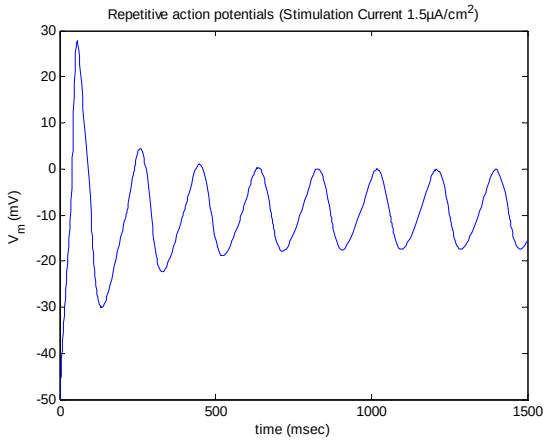

Figure 4 : Train of action potentials simulated for a stimulating current of 1.5 µA/cm^2.

Since relatively little is known about the myometrial ionic channels, some parameters used in this model are obtained from the literature. The others unknown have been chosen to fit the results with AP known characteristics of uterine cell at term.
A burst of action potentials is obtained in response to a long stimulation current (1.5 µA/cm^2). The difference of the first peaks of action potentials is due to calculation artefacts. So the system takes a certain time to stabilize. The system stability could be noticed in figure (4).
Moreover, the action potential peaks obtained by simulation was similar to the one observed in the literature [24] with a peak amplitude of about 30 mV and a peak duration of about 200 msec.
The amplitude of the plateau on which the spikes are superimposed also coincides with the data described in the literature [24].

Conclusion and perspectives

A combination of hypotheses and measured data found in the literature has been used to define this first model of uterine cell electrical activity.

Many questions concerning the uterine cell modelling approach remain to be answered which include the following. How much these hypotheses fit the physiologic parameters of a uterine cell? May this model be able to represent the different forms of uterine cell electrical activity observed during pregnancy? How important is sodium current and its implication in the model? How important is T-type Ca^{2+} current to human myometrium, especially its inactivation at -60 mV? How a more detailed inactivation calcium gating could be expressed? May this model simulate the slow wave uterine cell by changing the appropriate group of parameters? Until now, to answer these questions, there

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are no much details of ionic uterine cells channels available in the literature.
This model can be seen as a first step to approach a single uterine cell model. It takes in consideration the main myometrial ionic currents described in literature by using all the available parameters.
This model has been proved to be able to simulate a train of action potentials which parameters coincide with electrical activity observed at term.
Data used to define the individual mathematical models for the gating of each ionic current are either provided by experimental voltage clamp studies found in the literature or by hypothesis. Therefore, our first objective is to tune this model to be able to reproduce the electrical activity during pregnancy by using recent experiments on the properties of membrane currents and calcium handling in uterine cell. Studying and interpreting the roles of each ionic current and the variation of parameters such as ionic conductance, calcium variation parameters may be the first aim to simplify and validate the model. This would permit us to reproduce a wide variety of behaviour experimentally observed, as well as to develop a model of a single uterine cell electrical activity, and its propagation to the neighbouring cells and to the whole uterus. This is the first step needed to understand the relationships between the cellular and physiologic changes and the recorded global EMG characteristics, in order to detect preterm labour.

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