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Ionic Mechanism of Propagation in Human Purkinje fiber Cells: Role of Calcium Ions and Calcium Channels - a Simulation Study.

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Abstract

An important ailments that requires treatment by the physician is cardiac failure (“Heart Failure (HF)”), which usually decreases the contractility of the myocardium. The Purkinje Fiber Cells (PFC) augurs to be an important biological network of the human cardiac ventricular conduction system. It assuages to be tertiary pacemaker of the heart which synchronizes the ventricular conduction. This work analyzes the effects of calcium ionic mechanism of propagation using the Zhang model. The strength of contraction of cardiac muscle depends to a great extent on the concentration of calcium ions in the extracellular fluids. The calcium ions also play vital role in excitation, contraction coupling and cardiac muscle relaxation. The model attempts to increase the influx of calcium concentration (0.000156 mM) from the normal Ca^{2+} (0.000102 mM) value and also to increase the extracellular calcium concentration (3mM). The result show that the internal rise of calcium concentration increased excitability of PFC and maintain plateau potential throughout propagation. Similarly, the high calcium level in extracellular and low calcium level in intracellular decreased excitability of PFC and affect propagation diastole relaxation period. The Calcium channels appear to play a major role in sustaining conduction and propagation when low calcium level in intracellular fluids. The abnormal calcium concentration may affect the PFC excitability and Conduction velocity, which in turn can lead to cardiac ventricular arrhythmias.

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Keywords: Excitability, Extracellular fluid, Intracellular fluid, Action potential, Pacemakers, Calcium channels and Concentrations.

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1. Introduction

The heart is endowed with a special system for generating rhythmical electrical impulses to cause rhythmical contraction of the heart muscle and conducting these impulses rapidly through the heart. Another special importance of the heart is that all portions of the ventricles to contract almost simultaneously in additional support of Purkinje fibers [3]. In that Purkinje fibers are very large fibers, even larger than the normal ventricular muscle fibers, and they transmit action potentials at a velocity of 1.5 to 4.0 m/sec [8]. Electrical signals within biological organisms are in general driven by ions [2]. The most important cations for the action potential generation in cardiac cells are sodium (Na^+) and potassium (K^+) and calcium (Ca^{2+}) [3, 4]. The Calcium ions are normally very low concentration in the intracellular cytosol of virtually all cells in the body, at a concentration about 10,000 times less than that in the extracellular fluid. Many investigators have studied the way in which action potential (AP) propagates from PFC into the ventricular muscle cells and several hypotheses have been suggested [1]. Modeling of the electrical behavior of cardiac cells becomes necessary to study the determinants of the cardiac AP and furthermore to understand the interactions between different cardiac cells [2]. The mathematical model of the electrical AP of PFC is developed for different species (canine, rabbit, human and sheep) [25, 26 & 3]. Compare to other species, the human PFC AP lacks a numbers of characteristics, such as a longer Action Potential Duration (APD), lower plateau potential and less marked phase-1 notch than its ventricular counterpart [3]. It is possible that such differences in their APs are due to different properties of ion channels. In that transient outward k^+ current (I_{to}) channel kinetics as the main factor contributing to differences between human and canine PF APs. Excitability is important electro physiological property of excitable cell which respond on proper stimuli by generation of AP [5].

A mathematical model of the AP in human PFC that including, a high level of electrophysiological detail is developed by Zhang in 2009 [3]. The model is based on recent experimental data (Han et al. (2002)) on most of the major ionic currents and computationally cost-effective enough to be applied in large-scale spatial simulations for the study of cardiac arrhythmias. A description of I_{ion} for the human PF cell based on the model of the human endocardial cell by ten Tusscher et al. (2004) and ten Tusscher & Panfilov (2006) [23]. This model describing the properties of potassium currents in human PF cells based on the experimental data of Han et al. (2002) [3]. This model includes addition of two currents: a hyper polarization-activated current (I_{f}) and sustained potassium current (I_{sus}). The descriptions for the inward rectifier current (I_{K1}) and the transient outward current (I_{to}) were reformulated, maximum conductance of the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier potassium currents and the fast sodium current (I_{Na}) were altered because, these channels are distinctively different in channel kinetics and current densities between PF and ventricular cells [24]. Calcium plays two pivotal roles in cardiac excitation – contraction coupling [4]. Calcium concentration (Ca^{2+}) in cardiac cells drives myofilament activation and regulates ionic current that are responsible for normal cardiac rhythms [6]. A high calcium ion concentration, decrease the membrane permeability and simultaneously reduces its excitability. Therefore, calcium ions are said to be a Stabilizer [8]. The Ca^{2+} signal inducing contraction in cardiac muscle originates from two sources: Ca^{2+} enters the cell through voltage-dependent Ca^{2+} channels. This Ca^{2+} binds to and activates Ca^{2+} release channels (ryanodine receptors) of the sarcoplasmic reticulum (SR) through a Ca^{2+} -induced Ca^{2+} release (CICR) process. Ca^{2+} from both sources can then induce contraction [27]. Entry of Ca^{2+} with each contraction requires an equal amount of Ca^{2+} extrusion within a single heartbeat to maintain Ca^{2+} homeostasis and to ensure relaxation. Plasma membrane sodium-calcium (Na^+ - Ca^{2+}) exchanger is an essential component of Ca^{2+} signaling pathways in cardiac tissues [27]. Ca^{2+} is a major intracellular messenger, and nature has evolved multiple mechanisms to regulate Ca^{2+} levels. One of these mechanisms is Na^+ - Ca^{2+} exchanger. The Na^+ - Ca^{2+} exchanger is a transporter catalyzing the counter transport of three Na^+ for one Ca^{2+} . Detailed analysis of exchanger function has also been aided by the giant excised patch technique developed by Hilgemann [28]. More recently, several investigators have implicated Ca^{2+} influx via the Na^+ - Ca^{2+} exchanger as an important component of excitation-contraction coupling without invoking a subsarcolemmal accumulation of internal Na^+ [27].

The energy for net Ca^{2+} transport by the Na^+ - Ca^{2+} exchanger and its direction depend on the Na^+ , Ca^{2+} and K^+ gradients across the plasma membrane, the membrane potential, and the transport stoichiometry [29]. A large

signal-to-background ratio $\{\Delta[\text{Ca}^{2+}]_i/[\text{Ca}^{2+}]_{i(\text{rest})}\}$ can be generated with only a relatively small increment in the absolute amount of Ca^{2+} added to the cytosol [30]. The strength of contraction of cardiac muscle depends to a great extent on the concentration of calcium ions in the extracellular fluids. High extracellular $[\text{Ca}^{2+}]_o$ can also modifying the gating of all voltage dependent ion channels by reducing surface potential and high intracellular $[\text{Ca}^{2+}]_i$ in the PFC having the principle of increase excitability, but this effect has been less well documented experimentally [4]. $\text{Na}^+ - \text{Ca}^{2+}$ exchanger enhanced activity is responsible for the life threatening arrhythmias [7]. In this paper, simulation study made on how voltage-dependent Ca^{2+} channels and $\text{Na}^+ - \text{Ca}^{2+}$ exchanger has effects on the action potential and its propagation and calcium ion channels characteristics.

2. Methodology

In this paper, Zhang model of human Purkinje fiber cell is used for simulation analysis [3]. In general the electrophysiological behavior of a single cell can hence be described with the following differential equation.

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

Where,

C_m	-Membrane Capacitance in (μF)
V	-Membrane Potential in (mV)
t	-Time in (s)
I_{ion}	-is the sum of the transmembrane Ionic currents (pA/pF)
I_{stim}	-is an externally applied stimulus Current in (pA/pF)

Simulation analysis made on Zhang model of human PFC which dynamic membrane potential is given by equation.

$$dV/dt = - (I_{\text{ion}}) \quad (2)$$

Where, I_{ion} is given by summation of the number of ionic currents.

$$I_{\text{ion}} = I_{\text{kr}} + I_{\text{ks}} + I_{\text{kl}} + I_{\text{to}} + I_{\text{sus}} + I_{\text{Na}} + I_{\text{b,Na}} + I_{\text{Ca,L}} + I_{\text{b,Ca}} + I_{\text{NaK}} + I_{\text{NaCa}} + I_{\text{p,Ca}} + I_{\text{p,K}} + I_f \quad (3)$$

L-type Ca^{2+} ($I_{\text{Ca,L}}$), transient outward k^+ (I_{to}), rapid and slow delayed rectifier k^+ (I_{kr} & I_{ks}), Time-independent potassium current (I_{kl}), fast Na^+ (I_{Na}), $\text{Na}^+ - \text{Ca}^{2+}$ exchanger (I_{NaCa}), $\text{Na}^+ - \text{k}^+$ pump (I_{NaK}), and background Na^+ ($I_{\text{b,Na}}$), sarcolemmal calcium pump ($I_{\text{p,Ca}}$), background Ca^{2+} ($I_{\text{b,Ca}}$), sustained current (I_{sus}), Hyperpolarization-activated current (I_f) and potassium pump ($I_{\text{p,K}}$).

Abbreviation	Definition
PFC	Purkinje Fibre Cells
mM	milli Molar
s	seconds
mV	milli Volts
HF	Heart Failure
APD	Action Potential Duration
AP	Action Potential
$[\text{Ca}^{2+}]_o$	Extracellular Calcium Concentration
$[\text{Ca}^{2+}]_i$	Intracellular Calcium Concentration
DA	Depolarization Amplitude
$\text{Na}^+ - \text{Ca}^{2+}$	Sodium-Calcium exchanger($\text{Na}^+ - \text{Ca}^{2+}$)

Table 1: List of Abbreviation

3. Results and Discussions

Of the ions involved in the intricate workings of the cardiac cells, calcium is considered perhaps the most important. The normal physiological condition PFC having extra cellular $[Ca^{2+}]_o$ and intracellular $[Ca^{2+}]_i$ fluid calcium concentration levels as 2mM and 0.000101mM respectively. Under this condition PFC model is simulated up to 3 s, whose AP, L-type Ca^{2+} current ($I_{Ca,L}$), and $Na^+ - Ca^{2+}$ current (I_{NaCa}) are shown in Fig.1 (thin line), Fig.2 (thin line) and Fig.4 (thin line).

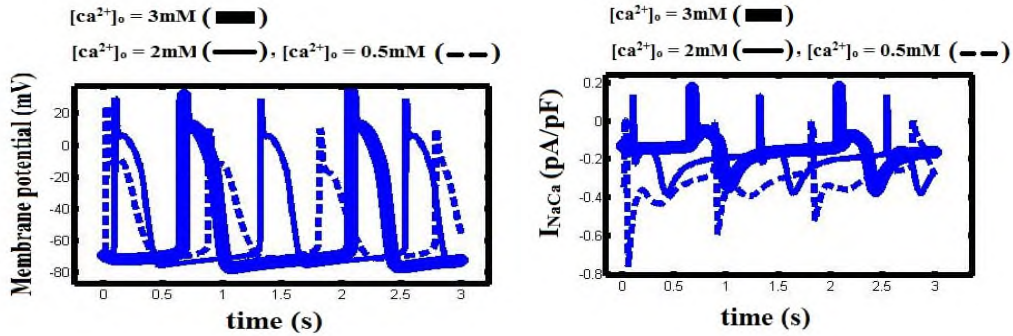


Fig.1: Action potentials generation in PFC (mV). $[Ca^{2+}]_o = 2mM$ its AP (thin line). $[Ca^{2+}]_o = 0.50mM$ and its AP (dot line). $[Ca^{2+}]_o = 3mM$ and its AP (thick line). Fig.2: $Na^+ - Ca^{2+}$ exchanger current (I_{NaCa}) generation in PFC (pA/pF). $[Ca^{2+}]_o = 2mM$ and its I_{NaCa} (thin line). $[Ca^{2+}]_o = 0.50mM$ and its I_{NaCa} (dot line). $[Ca^{2+}]_o = 3mM$ and its I_{NaCa} (thick line).

AP generation in PFC at normal calcium concentration level of $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ having maximum cycle length 1.2s, depolarization amplitude 29.3mV and its action potential duration (APD_{90}) 300ms (Fig.1- thin line).

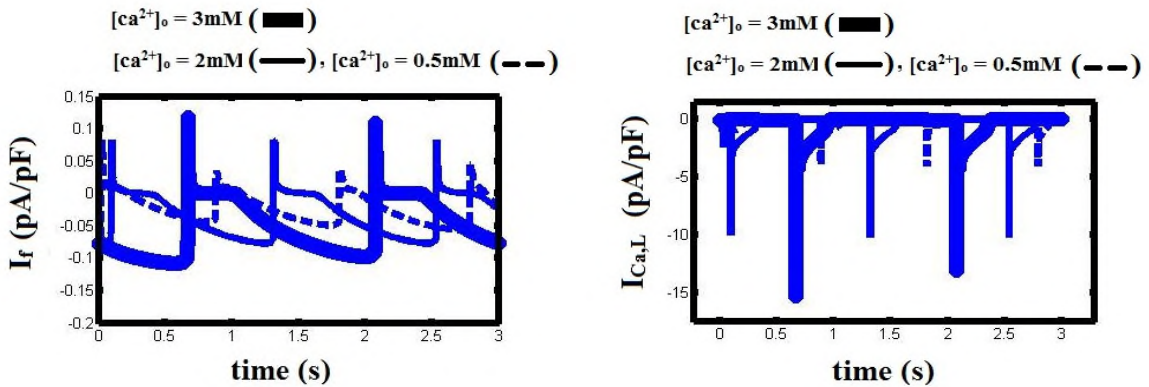


Fig.3: Hyperpolarization-activation current (I_f) generation in PFC (pA/pF). $[Ca^{2+}]_o = 2mM$ and its I_f (thin line). $[Ca^{2+}]_o = 0.50mM$ and its I_f (dot line). $[Ca^{2+}]_o = 3mM$ and its I_f (thick line). Fig.4: L-type Calcium current ($I_{Ca,L}$) generation in PFC (pA/pF). $[Ca^{2+}]_o = 2mM$ and its $I_{Ca,L}$ (thin line). $[Ca^{2+}]_o = 0.50mM$ and its $I_{Ca,L}$ (dot line). $[Ca^{2+}]_o = 3mM$ and its $I_{Ca,L}$ (thick line).

Intracellular calcium transient process takes place after 100ms at normal condition, because $I_{Ca,L}$ is increased towards more negative -10.2 (pA/pF) only on hundredth milliseconds (Fig.4- thin line). During this initial 100ms AP depolarization is happening due to Na^+ channels. L- Type Ca^{2+} channel raise the cytosolic Ca^{2+} level and produced enough threshold to open ryanodine channel. Plateau potential take place in AP of PFC is in the range of 5.5mV mainly due to $I_{Ca,L}$. Cytosolic Ca^{2+} level is also increase by Na^+-Ca^{2+} pump during reverse mode to maintenance of Ca^{2+} concentration in the sacroplasmic reticulum and cardiac muscle relaxation. I_{NaCa} peak is in the range of 0.15 (pA/pF) at normal PFC (Fig.2- thin line).

The strength of contraction of cardiac muscle depends to a great extent on the concentration of calcium ions in the extracellular fluids. The main focus of this work is to analyse the effects of calcium ions in extracellular fluids alters the excitation of AP. The role of $[Ca^{2+}]_o$ is to determine by reducing $[Ca^{2+}]_o$ to 75% (0.50 mM) from its normal physiological level and the model is simulate up to 3s. AP generation shows (Fig.1-dot line), cycle length decreases to 0.7s. Additionally, it decreases depolarization overshoot amplitude which various from 24.8mV-10.6mV, shortens relaxation period and also decreases the plateau potential of the APs.

This low $[Ca^{2+}]_o$ increase automaticity (excitability) is due to a shortening of the APD as a consequence of the loss of the AP plateau potential phase throughout the propagation, which is similar to previous experimental observations in peripheral rabbit SA node cells, in which the application of nifedipine, a inhibitor of $I_{Ca,L}$, shortened the rabbit SA node APD and accelerate its pacemaking activity (excitability) [31]. I_{NaCa} at level $[Ca^{2+}]_o = 0.50mM$ is shown in (Fig.2- dot line). In that Na^+-Ca^{2+} exchanger mechanism of calcium ions removal is very low, why because Ca^{2+} influx through L- type calcium channel into subspace or diadic space (SS) is not to provide enough thresholds to activate sacroplasmic reticulum ryanodine receptor (RyRs) channels that's why intracellular calcium transient (calcium induced calcium release- (CICR)) process does not increase the cytosolic calcium concentration. To balance $[Ca^{2+}]_o$, Na^+-Ca^{2+} exchanger removes one Ca^{2+} ions from intracellular to extracellular with counter transport of three Na^+ ions. This rapid increase inflow sodium ion through Na^+-Ca^{2+} pump during time course causes early rise in the AP depolarization. This low cytosolic calcium level affects the diastolic relaxation. As long as propagation execute, I_{NaCa} rapidly decreasing because of this low $[Ca^{2+}]_o$. Hyperpolarization-activated current (I_f) in action potential to play an important role in producing spontaneous diastolic depolarization leading to automaticity in particular cardiac cells [32]. I_f control and abnormal is shown in (Fig.3- (thin line and dot line)).

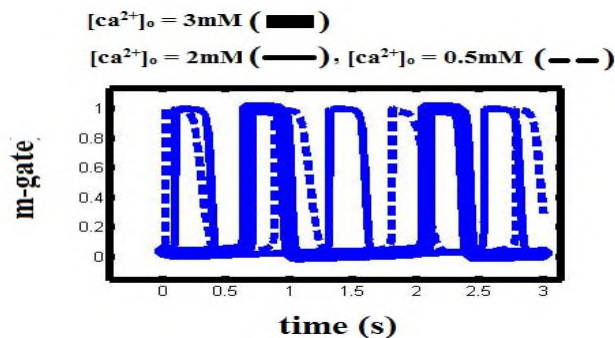


Fig.5: Sodium activation gate (m-gate) response in PFC. $[Ca^{2+}]_o = 2$ mM and its m-gate activation response (**thin line**). $[Ca^{2+}]_o = 0.50$ mM and its m-gate activation response (**dot line**). $[Ca^{2+}]_o = 3$ mM and its m-gate activation response (**thick line**).

I_f provides evidence to support the hypothesis that I_f is the primary factor responsible for generating pacemaking activity (Fig.3-thin line) [33]. But in (Fig.3- dot line) I_f time course is greatly reduce (~0.7s) by low cytosolic Ca^{2+} level and inflow sodium ion rise due to Na^+-Ca^{2+} exchanger accelerates pacemaking activity very early and its current rapidly decrease throughout propagation. This morphology changes is due to low $[Ca^{2+}]_o$, Which means I_f disturb the relaxation period and provide diastolic depolarization leading to automaticity in action

potential generation very early. These abnormal extra cellular calcium level, reduced the membrane potential cycle length (relaxation period) which means low $[Ca^{2+}]_o$ increase excitability of PFC but does not synchronization with ventricular contraction and relaxation. The role of $[Ca^{2+}]_o$ is also to determine by increasing $[Ca^{2+}]_o$ to 50% (3mM) from its normal physiological level and the model is simulated up to 3 s. AP generation shows (Fig.1-thick line), cycle length increase to 1.9s. Additionally, it increases depolarization overshoot amplitude 32.5mV, long relaxation period and also increases the plateau potential of the APs. AP depolarization phase taking more time (~500ms) and intracellular calcium transient process also takes more time (~650ms). This initial depolarization delay is due to high calcium ions in extracellular, block the opening rate of sodium activation gate (m) as shown in (Fig.5-thick line). This high $[Ca^{2+}]_o$ also increase the Na^+ reverse potential (E_{Na}), which gives delays to AP depolarization phase.

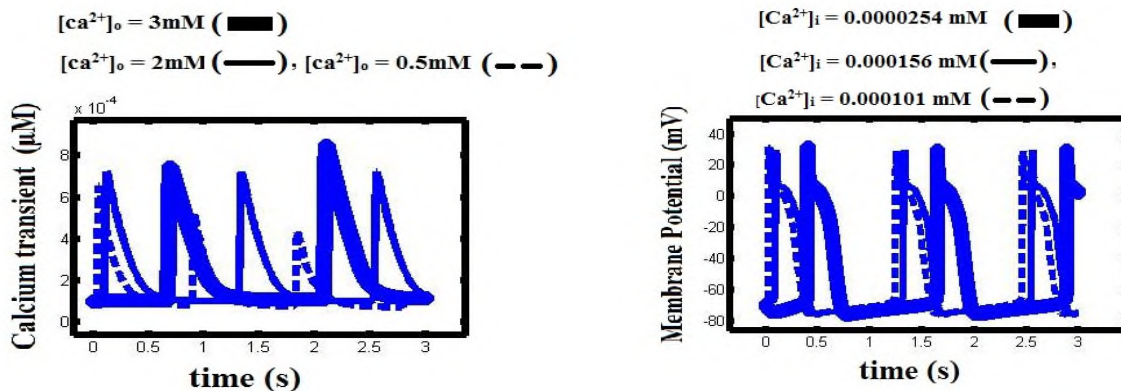


Fig.6: Calcium transient ($[Ca^{2+}]_i$) response in PFC (μM). $[Ca^{2+}]_o = 2 mM$ and its $[Ca^{2+}]_i$ transient (thin line). $[Ca^{2+}]_o = 0.50 mM$ and its $[Ca^{2+}]_i$ transient (dot line). $[Ca^{2+}]_o = 3 mM$ and its $[Ca^{2+}]_i$ transient (thick line). Fig.7: Action potentials generation in PFC (mV). $[Ca^{2+}]_i = 0.000101 mM$ its AP (thin line). $[Ca^{2+}]_i = 0.000156 mM$ and its AP (dot line). $[Ca^{2+}]_i = 0.0000256 mM$ and its AP (thick line).

Systolic Ca^{2+} transient is the spatial and temporal sum of such local Ca^{2+} releases (L-type Ca^{2+} channel release and Ca^{2+} RyRs channels release). Intracellular calcium transient delay (~650ms) is due to time take to activate the L-type Ca^{2+} channel activation gate (d-gate). Such a delay intracellular calcium transient process (accumulate Ca^{2+} in SS and that generates CICR process) at high $[Ca^{2+}]_o$ is clearly shown in (Fig.6-thick line). Diastolic relaxation period is increase more (~100ms) compare with normal PFC I_f . I_f shows noticeable change in diastolic relaxation period (Fig.3-thick line). I_{NaCa} at level $[Ca^{2+}]_o = 3 (mM)$ is shown in (Fig.2-thick line). In that Na^+Ca^{2+} pump is a transporter catalyzing the counter transport of three Na^+ for one Ca^{2+} . High calcium ions in extracellular, block the opening rate of sodium activation gate (m) up to 500ms, during this time interval Na^+Ca^{2+} pump also does not provide the exchange removal ionic mechanism. These excess extra cellular calcium level, increase the membrane potential cycle length which means high $[Ca^{2+}]_o$ take initially more time to reach depolarization threshold level, thereby it reduce excitability of PFC. The role of $[Ca^{2+}]_i$ is to determine by increasing $[Ca^{2+}]_i$ to 50% (0.000156 mM) from its normal physiological level and the model is simulated up to 3 s. AP generation shows (Fig.7-dot line), cycle length decrease to 1.1s. Additionally, it increase depolarization overshoot amplitude 31.5 mV. The AP generation does predict plateau potential throughout the propagation. This high $[Ca^{2+}]_i$ in the cytosol is to maintain the phase 2 plateau potential because, it provide enough threshold to release intracellular calcium transient (to open ryanodine channel).

These excess intra cellular calcium level, reduce the membrane potential cycle length which means high $[Ca^{2+}]_i$ increase excitability of PFC but does not synchronization with ventricular relaxation. In intracellular fluid calcium concentration level is decreased to 75% to its original concentration level ($[Ca^{2+}]_i = 0.0000254 mM$) and the model is simulated up to 3 s. Action potential generation shows (Fig.7-thick line) having maximum cycle length 1500ms and its depolarization amplitude various from 31.5mV - 29.4mV. These abnormal intra cellular calcium level, increased the membrane potential cycle length which means low $[Ca^{2+}]_i$ take initially more time to reach depolarization threshold level and repolarization period thereby it reduce excitability of PFC. So the calcium

concentration effects in PFC have been well document by simulation results and its shown very clearly in table 2.

Table 2: Different levels in intra and extra cellular calcium concentration and its characteristics of PFC.

S.No	[Ca ²⁺] _o (mM)	[Ca ²⁺] _i (mM)	AP Cycle Length (ms)	DA (mV)	Results
1	2	0.000101	1200	29.3	Normal Action Potential
2	0.50	0.000101	700	24.8-10.6	Increased Excitability and does not predict plateau potential
3	2	0.000156	1100	31.5-29.5	Increased Excitability and predict plateau potential and CICR
4	3	0.000101	1900	31.5-32.5	Depolarization delay and calcium transient delay
5	2	0.0000254	1500	31.5-29.4	Reduced Excitability, Depolarization delay and increase relaxation period

4. Conclusion

In this simulation analysis, the interior characteristics of ions channels, ions pump and cell membrane inactivate (or) activate gates can be understood clearly. Experimentally less documented of particular biological cell functions and characteristics are improved with help of simulation analysis. Low calcium level in extracellular and high calcium level in intracellular increased excitability of PFC and maintain plateau potential throughout propagation. Similarly, high calcium level in extracellular and low calcium level in intracellular decreased excitability of PFC and affect propagation diastole relaxation period. The abnormal calcium concentration affected the PFC excitability.

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