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Intracellular Calcium Oscillations

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1 Introduction

It is widely believed that oscillations in the concentration of cytoplasmic free Ca^{2+} in the cell are a signalling mechanism at the cellular level (Thomas et al. 1996). The signal is thought to be the frequency of the oscillations. Waves of increased Ca^{2+} also travel within and between cells, thus allowing the co-ordination of cellular activity over regions much larger than that of a single cell.

Pancreatic acinar cells are cells in the pancreas which synthesize the enzymes and zymogens required for digestion. They are formed into grape-like shapes that are known as acini (acinus sing.). The cells of a pancreatic acinus form a small tube in the centre, from which all the secretions travel into larger channels, eventually reaching the pancreatic duct. Calcium waves have been observed travelling around acini (Yule et al. 1996). The function of this intercellular Ca^{2+} wave is not entirely clear, although it appears to increase the efficiency of enzyme secretion of the acinus, presumably by co-ordinating the secretion of each individual cell.

A variety of agonists have been observed to stimulate Ca^{2+} oscillations. These agonists increase the calcium sensitivity of the endoplasmic reticulum (ER) (reticulum meaning storage place, endoplasmic meaning inside the cytoplasm). With the presence of enough calcium in the cytoplasm, this increased calcium sensitivity causes calcium-induced calcium release (CICR) from the ER. After the calcium in the ER is depleted, the rate pumping of calcium back into the ER is greater than the rate of CICR. Therefore the ER are replenished with calcium. If the agonist is still present and the concentration of other important substances are in the correct range, CICR will again deplete the calcium in the ER and thus the cycle repeats. The nature of intracellular calcium oscillations differs between the various agonists. For example some cause sinusoidal oscillations over small periods of time, and others cause sudden spikes of calcium concentration at longer time intervals. Calcium is involved in the movement of muscles, secretion of various proteins and fluids and perhaps some other functions. So these differing oscillation types allow these different processes to occur.

Various models have been constructed to simulate oscillations caused by individual agonists. We will consider two of these models, with the intention of combining these models in the future. The two under consideration model oscillations caused by the agonists: acetylcholine (ACh), and caffeine.

ACh diffuses through the extra-cellular space and attaches to the cell membrane by means of a receptor on the cell surface. Once this receptor has ACh bound, it initiates a series of reactions that end in the production of 1,4,5-trisphosphate (IP_3), which is free to diffuse through the cellular cytoplasm. On the surface of the intracellular calcium stores are receptors (called IP_3 receptors) which IP_3 can attach to. When IP_3 is attached to a receptor, the receptor acts as a Ca^{2+} sensitive gate (allowing CICR from calcium stores). The behaviour of these receptors/gates determines the nature of the intracellular calcium oscillations, and so mathematical models of these oscillations are based upon the behaviour of these IP_3 receptors.

It has also been observed that caffeine can cause intracellular calcium oscillations. Furthermore these oscillations can be stopped by the introduction of ryanodine. On the surface of the ER are receptors (known as ryanodine receptors) which ryanodine attaches to. Since ryanodine stops calcium oscillations caused by caffeine, we can deduce that the ryanodine receptors are involved in calcium release. It has been observed that ryanodine receptors release calcium when enough calcium is in the cytoplasm, in other words ryanodine receptors facilitate CICR. As mentioned before these oscillations occur with the

presence of caffeine, therefore since we know that CICR is occurring, we can deduce that caffeine increases the sensitivity of ryanodine receptors to calcium. The mathematical models simulating these oscillations are referred to as the ryanodine receptor model.

2 The IP_3 Receptor Model

ACh binds to a cell surface receptor thus activating a G-protein, which in turn activates phospholipase C (PLC), producing inositol 1,4,5-trisphosphate (IP_3) from phosphatidylinositol bisphosphate. IP_3 diffuses through the cell cytoplasm until it binds to an IP_3 receptor on the surface of the ER.

An IP_3 receptor is also a gate on the surface of the ER, so this binding is necessary for an IP_3 receptor to open. It consists of four identical independent sub-gates each with an IP_3 binding site and at least two Ca^{2+} binding sites, one for activation (opening) and one for inactivation (closing) of the IP_3 receptor. For an IP_3 receptor to be fully open each of the four sub-gates must be open. These sub-gates are open when there is IP_3 on the IP_3 site, Ca^{2+} on the activation site, and no Ca^{2+} on the inactivation site. Once an IP_3 receptor is open, then Ca^{2+} can leak out of the ER into the cytoplasm. Notice that the calcium is required for the IP_3 receptor to open. Therefore, as mentioned before, calcium release is induced by calcium (CICR). There is plenty of data that suggests that the time required for IP_3 receptor inactivation is slow in comparison to the time that is required for IP_3 receptor activation. This means that an IP_3 receptor can open quickly and stay open for a reasonable period of time (allowing Ca^{2+} to leak out), before inactivation by Ca^{2+} occurs. The models proposed in [2] and [5] by Sneyd et al. (1999, 2000) use this information.

Also on the surface of the ER are proteins called calcium ATPases, which pump Ca^{2+} into the ER. Thus once most of the IP_3 receptors are inactivated calcium pumps reduce the cytoplasmic calcium concentration. If the IP_3 concentration is high enough, then as cytoplasmic calcium decreases, more IP_3 receptors are opened allowing Ca^{2+} to be released as before. This in turn causes the cytoplasmic calcium concentration to increase. If the IP_3 concentration stays within a certain range, the above processes will repeat.

The Ca^{2+} oscillations of a cell can be influenced by extracellular release of Ca^{2+} from a neighbouring cell. The combined effect this neighbouring influence is that waves of Ca^{2+} propagate across cell neighbourhoods. These waves are thought to co-ordinate cells in the same neighbourhood. The example of waves causing co-ordination in pancreatic acinar cells was a case of IP_3-induced oscillations.

The IP_3 model we consider is a model proposed by Sneyd et al. (2000) in [5]. This model simulates experimental observations of IP_3-induced calcium oscillations in pancreatic acinar cells. For each of the IP_3 receptor sub-gates there is a shut state, an open state, and an inhibited state. Let c represent cytoplasmic calcium concentration with x, y and z representing the percentage of IP_3 receptor sub-gates which are shut, open, and inactivated, respectively. The differential equations below are based on Diagram 1.

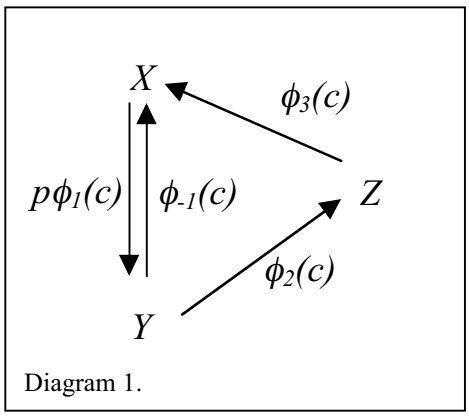

Diagram 1.

$$\frac{dx}{dt} = \phi_{-1}(c)y - p\phi_1(c)x + \phi_3(c)z,$$

$$\frac{dy}{dt} = p\phi_1(c)x - \phi_{-1}(c)y - \phi_2(c)y,$$

$$z = 1 - x - y$$

where

p = concentration of IP3

$$\phi_1(c) = \frac{r_2 c}{R_1 + c},$$

$$\phi_{-1} = \frac{k_{-1} R_3}{c + R_3},$$

$$\phi_2(c) = \frac{k_2 R_3 + r_4 c}{R_3 + c},$$

$$\phi_3(c) = \frac{k_3 R_5}{R_5 + c}.$$

These formulas are not just a 'rough hack', but for details on their derivation refer to (Sneyd 2000). Assuming the change in cytoplasmic calcium levels is the sum of fluxes across the membrane and ER membrane we derive the simple formula,

$$\frac{dc}{dt} = J_{receptor} - J_{pump} + J_{leak},$$

where $J_{receptor}$ is the calcium flux through the IP_3 receptor, J_{pump} the flux through the calcium ATPase pumps, and J_{leak} is the flux through the cell membrane. While J_{leak} is a constant, J_{pump} and $J_{receptor}$ are given by

$$J_{pump} = \frac{V_p c^2}{K_p^2 + c^2},$$

$$J_{receptor} = k_f y^4,$$

where V_p, K_p and k_f are constants. This gives J_{pump} as a non-negative (since Ca^{2+} concentration can not be negative) monotonically increasing function of calcium concentration and asymptotically approaches V_p. In other words the higher the calcium concentration the more is pumped back into the ER. There are not an infinite number of pumps, and they can not pump infinitely fast, therefore there is a limit to how much Ca^{2+} can be pumped. The 'c^2' terms reduce the second derivative maximum so that the transition from $J_{pump} = 0$ to $J_{pump} \approx V_p$ is not too sudden. Since there are four sub-gates, $J_{receptor}$ is the maximum leak rate (k_f) multiplied by the percentage of receptors in which all four of the independent identical sub-gates are open (y^4). This system of equations is non-linear. Therefore there does not exist an exact solution and they have to be solved by numerical methods.

Numerical Solution

We have implemented this model numerically using Euler's method with a C program. When the IP_3 concentration is 0.44μM (figure 1), the model gives steady sinusoidal oscillations with a ~10-11 second period. Figure 2 shows the behaviour of the model over different IP_3 concentration levels. From this figure we can see there is a minimum and maximum IP_3 concentration which stimulates oscillations. Outside of these limits the calcium concentrations stabilise to a constant level. From Figure 2 we see that increased IP_3 causes an increase in frequency and amplitude.

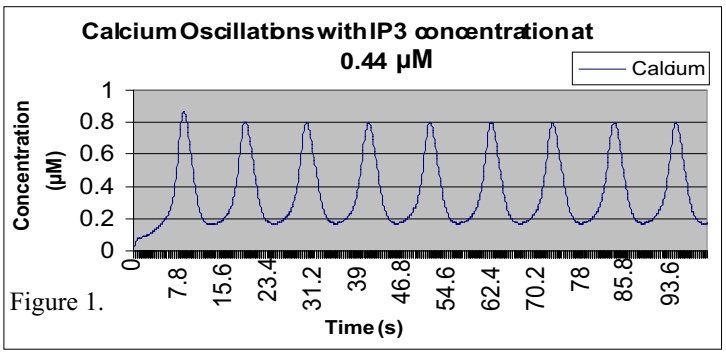

Figure 1.

$k_f = 28$	$J_{leak} = 0.2$
$V_p = 1.2$	$K_p = 0.18$
$k_3 = 1$	$k_2 = 0.53$
$r_2 = 100$	$k_{-1} = 0.88$
$R_1 = 6$	$r_4 = 20$
$R_5 = 1.6$	$R_3 = 50$

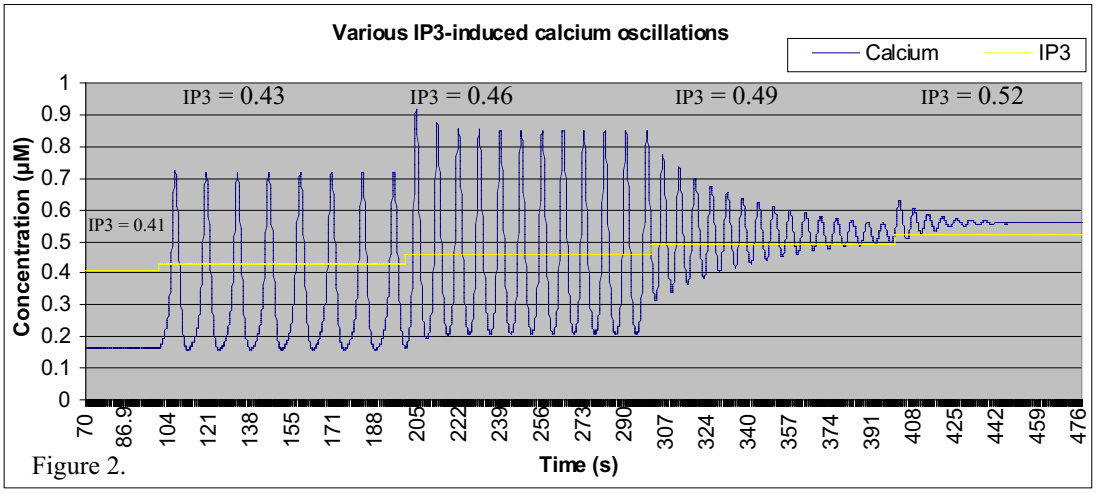

Figure 2.

3.1 Ryanodine Receptor Model

There are other ways in which calcium can be released from the ER other then IP_3 receptors. Ryanodine receptors have a similar function, structure, and sensitivity to calcium as IP_3 receptors do. Ryanodine receptors are completely disabled by ryanodine and activated with calcium. An increase in caffeine concentration increases the sensitivity of ryanodine receptors to calcium. This increases the possibility of CICR and therefore the possibility of calcium oscillations.

Friel's model assumes that there are four fluxes, 1) a leak from extracellular space into the cytoplasm (J_{L1}), 2) a flux from pumps pumping calcium from the cytoplasm into extracellular space (J_{P1}), 3) a leak from the ER into the cytoplasm (J_{L2}), and 4) a flux from pumps pumping calcium from the cytoplasm into the ER (J_{P2}). J_{L1} is proportional to the difference between the concentration of calcium across cell membrane. J_{P1} and J_{P2} are proportional to calcium concentration in the cytoplasm. J_{L2} is where the non-linearity comes into play. Similar to J_{L1}, J_{L2} is proportional to the difference of calcium concentrations across the ER membrane, except the co-efficient is not constant, but a function of cytoplasmic calcium concentration. More specifically we have,

$$J_{L1} = k_{L1}(c_i - c_o),$$

$$J_{P1} = k_{P1}c_i,$$

$$J_{L2} = k_{l2}(c_i - c_o),$$

$$J_{P2} = k_{P2}c_i,$$

where c_i is cytoplasmic calcium concentration, c_o is external calcium concentration (constant), c_s is calcium concentration inside the ER, k_{L1}, k_{P1} and k_{P2} are constants and,

$$k_{L2} = k_{L2}^{(0)} + \frac{k_{L2}^{(1)} c_i^n}{c_i^n + K_d^n}.$$

The coefficient k_{L2} is a key part of the model; the source of CICR. It is a sigmoidal function of calcium from $k_{L2}^{(0)}$ (from non-negativity of calcium concentration) to $k_{L2}^{(0)} + k_{L2}^{(1)}$ (the maximum leak rate). Increasing n increases the maximum of the first derivative (i.e. increases the slope of k_{L2}). The variable K_d is also known as the half maximal of the function because when c (calcium concentration) is equal to K_d, k_{L2} is half of the maximum leak rate. A drop in K_d has the effect of increasing the slope of k_{L2} and reducing the concentration of calcium required for CICR to begin. Both an increase in the slope and reduction of calcium required for CICR, increases the effect of calcium on CICR, which is what happens when caffeine concentration is increased. Thus drop in K_d models an increased extracellular (and intracellular) caffeine concentration.

The change in calcium concentration is given by,

$$\frac{dc_i}{dt} = -[J_{L1} + J_{P2} + (J_{L2} + J_{P2})\gamma],$$

$$\frac{dc_s}{dt} = [J_{L2} + J_{P2}].$$

Note that the coefficients k_{P2} and k_{L2} are inversely proportional to the volume of the ER. Likewise k_{L1} and k_{P1} are inversely proportional to the volume of the cytoplasm. Therefore the constant _ (which is the ratio of these volumes) converts J_{L2} and J_{P2} to the same proportion as J_{L1} and J_{P1}. Since k_{L2} is a sigmoidal function of c_i, the equations for c_i and c_o are non-linear and have to be solved numerically.

3.2 Numerical Solution

Similarly to the IP_3 solution, Euler's method was used in a C program. Again, like IP_3-induced oscillations in figure 1, the caffeine-induced oscillations in figure 3 are quite steady. A large initial uptake of calcium is evident immediately after K_d was changed to 0.4, followed by oscillations with a period of ~40 seconds. From figure 4 we can see that there is a minimum and maximum concentration of caffeine required for oscillations to occur. It is also evident from this figure that as the concentration of caffeine is decreased, the frequency of the oscillations decreases while amplitude increases.

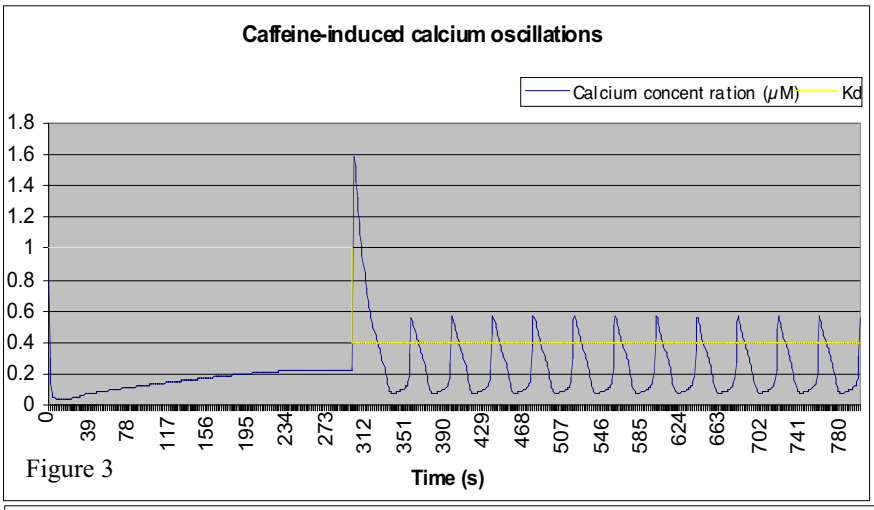

Figure 3

$k_{L1} = 5 \times 10^{-5}$
$k_{P1} = 0.132$
$k_{L2}^{(0)} = 0.054$
$k_{L2}^{(1)} = 2.4$
$k_{P2} = 3.78$
$n = 3$
_ = 0.24

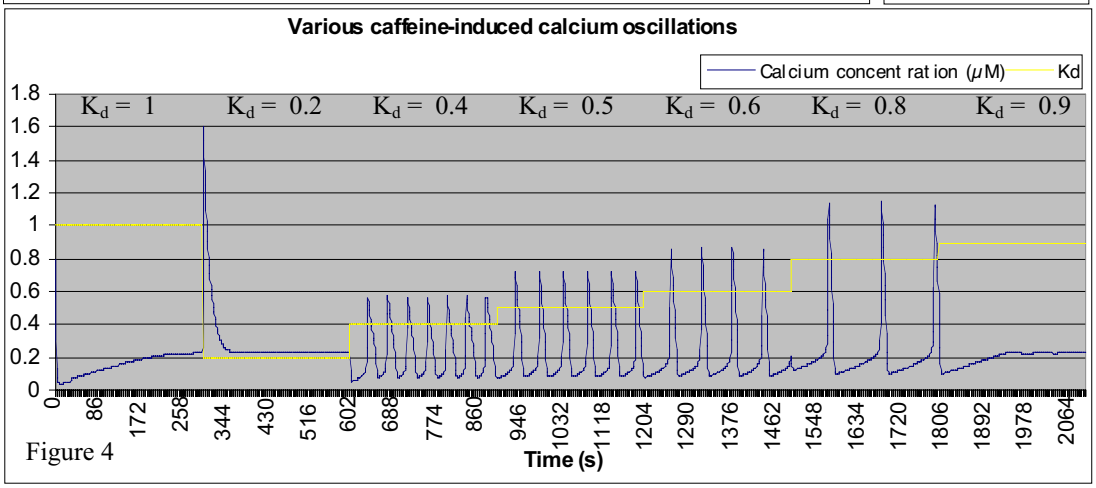

Figure 4

4 Combining Models

Obviously the two models mentioned above only consider calcium oscillations caused by one of the two receptor-types each. In reality these oscillations caused by caffeine, ACh, and CCh can occur at the same time. This is because the mechanisms behind the two models exist in the same cells. A generalized model must take both mechanisms into account. By successfully combining the IP_3 and caffeine models we can create such a generalized model. We can then observe the behaviour of oscillations in this generalized model, hoping to see a similarity with what really happens. This will help with the understanding intercellular waves of calcium, and the cellular co-ordination that they create. At present, we are still combining these models with a hybrid C program. The general idea will be to *a)* have a new function J_{L2}* which is the sum of $J_{receptor}$ and J_{L2} *b)* instead of using a linear J_{P2}, as in the ryanodine model, use one similar to J_{pump} in the IP_3 model. This will assume both IP_3 and ryanodine receptors use the same Ca^{2+} store. It shall be interesting to see what this hybrid model will show us about inter-cellular calcium waves.

References

[1] Friel, D. D., (May 1995),"$[Ca^{2+}]_i$ Oscillations in Sympathetic Neurons: Experimental Test of a Theoretical Model", Biophysical Journal Volume **68**, 1752-1766.

[2] LeBeau, Andrew P., David I. Yule, Guy E. Groblewski, James Sneyd, (June 1999), "Agonist-dependent Phosphorylation of the Inositol 1,4,5-Trisphosphate Receptor *A Possible Mechanism for Agonist-specific Calcium Oscillations in Pancreatic Acinar Cells*", J. Gen. Physiol. Volume **113**, 851-871.

[3] Mckenzie, A., J. Sneyd, (1998),"On The Formation and Break-up of Spiral Waves of Calcium", International Journal of Bifurcation and Chaos, Vol. **8**, No. 10, World Scientific Publishing Company.

[4] Moran, Laurence A., K. Gray Scrimgeour, H. Robert Horton, Raymond S. Ochs, J. David Rawn, (1994), "Biochemistry" Second Edition, Prentice Hall Upper Saddle River, NJ 07458.

[5] Sneyd, J., A. Lebeau, D. Yule, (24 March 2000), "Travelling Waves of Calcium in Pancreatic Acinar Cells: Model Construction and Bifurcation Analysis", Physica D, in press.

[6] Thomas, A. P., G. S. J. Bird, G. Hajn_csky, L. D. Robb-Gaspers and J. W. J. Putney, (1996) "Spatial and temporal aspects of cellular calcium signaling", FASEB *Journal* 10: 1505-1517.

[7] Yule, D. I., A. M. Lawrie and D. V. Gallacher, (1991) "Acetylcholine and cholecystokinin induce different patterns of oscillating calcium signals in pancreatic acinar cells.", *Cell Calcium*. 12: 145-151.

[8] Yule, D. I., E. Stuenkel and J. A. Williams, (1996) "Intercellular calcium waves in rat pancreatic acini: mechanism of transmission", *American Journal of Physiology (Cell Physiol.)*. 271: C1285-C1294.