The Calcium Channel

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Calcium has an unquestionable messenger role in muscle contraction and innumerable physiological processes like blood clotting and coagulation, cellular adhesion and integrity, membrane stability, bone and teeth formation, enzyme activity, control of certain aspects of cyclic nucleotide metabolism, mediation of some actions of prostaglandins, cell division and motility, glandular and other cellular secretions, neuronal transmission, and possibly behaviour (5, 6, 30, 33).

Studies on the calcium-channel has gained impetus in the last decade from the development of a new class of structurally diverse group of organic compounds known as calcium-channel antagonists, calcium antagonists, calcium-entry blockers, slow-channel inhibitors, or calcium-entry antagonists, used indispensibly by clinicians for various cardiovascular disorders (27,38,40).

The role of calcium was studied a century ago by Sidney Ringer (32). He observed that calcium-free saline led to cardiac arrest of isolated frog heart. Mines (23) observed that calcium withdrawal mainly affected the mechanical performance without affecting the bioelectric processes of ventricular excitation. Later, Sandow (34) observed that an action potential in the muscle promotes the entry of calcium into the myoplasm which leads to mechanical activity. The unique role of calcium has been attributed to its ionic properties that appear to allow selective and reversible interactions with anionic groups abundant in the proteins of living systems.

Normally, the calcium ions of unstimulated cells is within the range of 5×10^{-8} to 5×10^{-7} M, while that of the extracellular fluid is 10^{-3} to 10^{-2} M. On electrical or chemical stimulation, the intracellular calcium ions increase by one to two orders of magnitude either as a result of calcium influx across the cell membrane, or through the release of calcium from intracellular stores, or both. The density of functional calcium channels per cultured rat cardiac cell is 2000 to 10,000, and the number of ions flowing through each channel is approximately 3×10^{-6} per second (30).

Within the cell, the targets for calcium are a homologous group of calcium binding proteins, including parvalbumins, troponin C, and calmodulin, which confer the induced events (6, 18, 21, 24). The sources of calcium are either the extracellular compartments like the basement membrane, ground substance, and extracellular fluids, or the intracellular calcium storage pools like mitochondria, sarcoplasmic-reticulum, nucleus, inner-aspect of plasma-membrane, or secretory vesicles.

Glandular and smooth-muscle have limited endoplasmic calcium and their contractility is dependent on extracellular calcium, while cardiac-muscle contraction utilizes both intra-and extracellular calcium. On the contrary, skeletal-muscle contractions are unaffected from extracellular calcium fluctuations due to their well developed intracellular calcium stores.

At rest, intracellular calcium haemostasis is maintained by several mechanisms. The cell membrane is relatively impermeable to calcium at rest besides a certain amount leaking into the cell due to high concentration gradient inbetween the extra- and intracellular fluids. ATP-driven calcium pumps on the cell membrane reduce the free calcium level in the cytoplasm (36). Similar intracellular pumps sequester calcium to the intracellular organelles. A calcium-sodium counter transport lowers cellular calcium level (4, 41). An ATP-dependent calcium haemostasis has also been observed in erythrocytes (36).

There is now sufficient evidence for the existence of discrete ionic pathways much more selective for calcium ions than for sodium or potassium ions (25). The calcium channels have been identified by binding studies in the aorta (3, 19), intestine (19), cardiac and vascular smooth-muscle (3, 17, 35, 37, 41), and brain (9, 13, 17, 29). (Also 10, 12, 42). They have been found to be temperature dependent (1). They have also been solubilized and found to contain a glycoprotein (11).

BAY K 8644 (17) and CGP 28382 (44), both dihydropyridines activate the calcium channels. Maiotoxin, a marine toxin also stimulates calcium influx (26). These agonists are competitively inhibited by the organic calcium antagonists, including the dihydropyridines.

Som sodium ions can move through the calcium-channels. This is probably due to a binding site for divalent cations within the channels or the presence of some tetrodotoxin-insensitive pathway.Despite the apparent incomplete selectivity of calcium-channels for calcium, they are still amongst the most selective channels in excitable membranes (14,25,30,45). The calcium-channels require energy in the form of ATP for their maintenance, and are the main pathway for sodium entry in the embryonic heart (25). Some potassium ions can move outwards through the calcium-channels (22).

The calcium-channels are affected by inorganic cations like barium and strontium, which can substitute for calcium as the main charge carrier, and nickel, cobalt, and lanthanum, which are inhibitory (4, 14, 25, 43). This property presumably arises from their rather nonselective ability to substitute for calcium-binding sites. A cation-coordination site is a likely feature of all calcium-channels (14, 43).

Calcium influx may occur through two distinct pathways—one activated by membrane-depolarization or electromechanical coupling, known as potential-dependent channel (PDC), and the other by receptor-mediated events or pharmacodynamic coupling, known as receptor-operated channel (ROC) (2). Both ROC- and PDC-mediated events utilize the calcium from extracellular space, but the ROC-mediated events, in addition, also utilize the intracellular calcium.

In the PDC, calcium influx across the excited muscle is 'electrogenic', i.e., calcium ions, in addition to sodium and potassium, contribute to the changes in membrane-potential (30). A 23,000 dalton sarcolemmal lipoprotein called calciduction has been isolated, which may be the calcium-channel or one of its components (31).

An important feature of the PDC is that its conductance is controlled by two distinct activation and inactivation gates. In cardiac tissues the inactivation time for calcium (slow) channels are much longer than that of the sodium (fast) channels, following initiation of the cellular depolarization process (21). The calcium-channels are operated over a higher voltage range (-45 to -25 mV), than the sodium channels (-80 to -50 mV).

The PDC opens in bursts and the burst-lengths increase with voltage. The average duration of channel opening (approx. 1 ms) is moderately voltage dependent (30, 45). The PDC is probably composed of macromolecular protein that traverse the phospholipid bilayer, which comprises the sarcolemma and selectively permits ion fluxes. The channels are highly specific for a given ion species, and an aqueous 'pore' within each channel is probably provided with a 'selectivity-filter', which screens the passing ion. This hydrophilic screening mechanism is more efficient than that of either potassium or sodium channel. The channel may also have 'voltage-sensors' or voltage-dependent gating which determines the state of the channel gates (14, 43). The voltage-sensor may be a protein group with dipole properties which reacts to an electric field.

In the smooth-muscle, PDC-mediated contractile responses can be differentiated into fast, transient or phasic component, and slow, sustained or tonic component. The phasic component is related to calcium influx during action-potential and its importance lies in the regulation of short term changes in the contractile state. In the tonic component, smaller, graded depolarization occurs with the degree of contraction correlating to the degree of membrane-depolarization. The time and course of the tonic component depends upon the extracellular calcium concentration (14, 16, 39). The sustained membrane-depolarization activates calcium-channels which are biologically distinct from those in other muscles. The tonic and phasic components are associated with different cellular processes due to the observations that :

(a) the phasic component reaches its peak tension change more rapidly than the tonic component,

(b) the phasic component is less sensitive than the tonic component to the slow developing inhibitory effect of a high extracellular potassium concentration,

(c) the phasic component is more sensitive than the tonic component to the rapidly developing inhibitory effect of extracellular calcium ions (15), and

(d) the phasic component exhibits a greater sensitivity than the tonic component to the inhibitory effect of lanthanum ions.

The relationship of the calcium-channels to the receptors is still to be established. The relative long latent-period in the response shown by some smooth-muscles suggest some biochemical interactions. It is unclear whether different receptors operate discrete populations or common pools of channels. Different receptor stimulation may lead to

different channel stimulation (43). Various hypotheses have been proposed, as follows :

(a) Both types of channels may operate either separately or in a linked fashion. The PDC may change its voltage dependence and sensitivity to ROC.

(b) The entering calcium ions, known as 'trigger-calcium' during stimulation triggers the release of calcium from intracellular-loci or membrane, known as 'activator-calcium' (41). The activator-calcium may modulate the state of activation of the ROC such that greater the intracellular calcium release, greater the calcium influx through the ROC (4).

(c) Several different membrane proteins control calcium permeability, some of which respond principally to depolarization and some to hormonal agents. This is caused by increase in calcium permeability due to PDC or ROC mediated events which may be additive when the two procedures of activation are used simultaneously (4).

(d) The ROC is phosphorylated when active (channel available for opening and closing cycles) and dephosphorylated when inactive (28, 30).

(e) Adrenergic agonists recruit additional calcium receptors to produce their effects (4, 30).

(f) Histamine, through H_2-receptors, enhance calcium influx (8).

Two fundamentally different types of calcium-channels have also been distinguished physiologically by temperature-sensitivity (7).

Inactivation of the calcium-channels is by a voltage-dependent inactivation and an 'inactivation-process'. The inactivation-process depends directly on the passive flow of calcium current through the channel (14). Inactivation may also result from the reduced availability of the channel and not from the reduction of current (30).

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