Chapter II

Background Information

Calcium and cell function

The crucial role of calcium in the regulation of cell functions has been appreciated for a long time. However, in the last decade this field has undergone an explosive growth. This is so mainly because of the enormous advance in the knowledge of the biochemistry of the effects of calcium. It is made possible by the discovery of the widely distributed intracellular calcium-binding proteins that mediate most of the effects of calcium. The study of the cellular effects of calcium has become a central subject in physiology, biochemistry, and cell biology.

The messenger role of calcium

It is now well established that cells increase their cytosolic levels of calcium ions in response to many stimuli and that an increase is the primary event that triggers the cellular response. No case is known in which the response to a stimulus leads to a decrease in the cytosolic concentration of calcium ions (Kretsinger, 1981). Cytosolic calcium ions therefore acts as a "second messenger" that delivers to the effector system by the signal of the stimulus. In eukaryotic cells, the role of calcium ions as a second messenger is as important as the

more widely known messenger role of cAMP. The nucleotide and calcium ions may mediate responses to different stimuli, but frequently the responses to both are coordinated, and in some cases, cAMP and calcium ions affect in different ways in the same effector system. The suitability of calcium ions chemistry for the role of calcium ions as a second messenger as compared with the more abundant monovalent cations has been examined by Urry and coworkers (1982) who postulated that it derives from the greater restrictions for the movements of calcium ions across the cell membrane and its greater affinity for binding to protiens and polypeptides. The main experimental support for the role of calcium ions as a second messenger comes from studies that : (1) demonstrate a correlation between the changes in the cytosolic calcium ions and the time course of a stimulus, (2) show that a cellular response can be reproduced in the absence of its physiological stimulus by artificially raising the cytosolic level of calcium ions, and (3) show a direct dependence on calcium ions of an enzyme or other intracellular system.

The mechanisms of the increase of cytosolic calcium ions stimulated cell

Depending on the cell and on the stimulus, cytosolic calcium ions concentration may increase, either as consequence of the inflow of extracellular calcium ions or because calcium ions are released from intracellular stores (Somlyo, 1984). In the first case, the response to the stimulus disappears when the cells are suspended in a

calcium-free medium, whereas in the second case, the stimulus may be effective for long periods of time even in the complete absence of extracellular calcium ions. It seems likely that release from intracellular stores is the preferred mechanism in the large cells and when the whole cell must respond simutaneously.

Somlyo (1984) suggested the idea that in most cells the main intracellular store of messenger calcium ions is the endoplasmic reticulum rather than the mitochondria. This is in keeping with the fact that the cytosolic calcium ions concentration in stimulated cells is usually below that needed to switch on high-capacity and relatively low-affinity mitochondria calcium ions transport system.

: Calcium ion channels in excitable membranes

Regardless of the source, the flow of calcium ions into the cytosol that follows the stimulus is a net flow down an electrochemical potential gradient. In most cases, this probably is the consequence of a transient increase in the permeability to calcium ion store. Electrophysiological studies have shown unequivocally that the plasma membrane of cells that respond to stimuli with changes in membrane potential channels that selectively allow the entry of calcium ions down its electrochemical potential gradient (Reuter, 1983). These channels have two main functions : to allow the participation of calcium ions currents in the rising phase of the action potential; and to allow the inflow of extracellular calcium ions that leads to the

rise in cytosolic calcium ions concentration and the consequent cellular response to the stimulus.

Calcium ion channels are controlled by voltage-dependent gating, that is, their opening or closing kinetics are a consequence of changes in membrane potential. In resting cells, the channels are closed. The probability that a channel will open increase steeply with depolarization. The channels show both time- and voltage-dependent inactivation. Usually they open at more positive membrane potentials than the sodium ion channels which participate in the rising phase of the action potential. The calcium ion channels are one of the most selective of several kinds of channels which can be detected in excitable membrane. For example, the selectively for sodium ions of the sodium ion channels is at least one order of magnitude less than the selectively for calcium ions of calcium ion channels.

Although calcium ion channels are primarily regulated by the membrane potential, their properties are modulated by neurotransmitters, hormone, and drugs. In some cases, modulation seems to imply phosphorylation of the channels or of membrane proteins closely associated with it.

: Cell membrane phospho- and polyphosphoinositides and receptor-mediated calcium ions mobilization

A variaty of biologically active substances acting at

receptors on the plasma membrane provoke the increase in the turnover of membrane phosphoinositides (PI) and polyphosphoinositides (PIs). PI have one phosphate group in the inositol ring, whereas PIs have one or two additional phosphate groups. Polyphosphoinositides include phosphatidylinositol 4-phosphate(PIP) and phosphatidylinositol 4,5-bis-phosphate (PIP2) (Somlyo, 1984). The increase in turnover of these compounds is associated with the mobilization of intracellular calcium ions and not with the synthesis of cAMP. Stimulation of turnover dose require extracellular calcium ions and is unaffected by changes in intracellular calcium ion concentration.

Receptors involved in this type of response include the muscarinic receptor for acetylcholine, the alpha-1 adrenergic receptor, the thrombin receptor of platelets, and one type of receptor for vasopressin.

Calcium binding proteins : Calmodulin

The best-known and more generally distributed calcium binding proteins is calmodulin, which mediates calcium messages in most eukaryotic cells. The concentration in most tissues varies between 2 to 40 micromole/kg wet weight (Cheung, 1979; Klee et al., 1980). It is a small acidic protein (isoelectric point around 4), composed of a single polypeptide chain of 148 amino acid residues with a high content of alpha helix. Calmodulin is extremely resistant to heat, acid, and other treatments that promote protein denaturation. Since calmodulin has a

highly flexible tertiary structure, a feature which may be related to its ability to interact with very different target proteins (Klee et al., 1980). The first complete amino acid sequence of calmodulin was reported by Watterson et al. (1980)

Calcium regulate in endo- and exocytosis

Recent experiment evidence strongly suggests that calcium has an important role in the processes that lead to the formation of endo- and exocytic vesicles. Although little is known about the detailed biochemistry of these processes, it seems that the effects of calcium are mediated by the calcium-calmodulin complex. Endo-and exocytic vesicles have, in their external regions, sites that bind calmodulin or calcium-calmodulin. The effects of calcium and calmodulin are, at least in some cases, mediated by the phosphorylation of membrane vesicles. For example, the processes of exocytosis in the release of neurotransmitters from the nerve terminal at synapsis. In these structures, the depolarization was carried by the action potential opens voltage-dependent calcium channels in the nerve terminal allowing the entry of extracellular calcium ions with the consequent rise in cytosolic calcium ions (Llinas et al., 1981). Calcium and calmodulin bind to the synaptic vesicles and initiate a series of biochemical and morphological events whose ultimate result is that vesicles fuse with the plasma membrane and release their contents into space that nerve ending and the postsynaptic region (Shulman and Greengard, 1979; Chiese and Carafoli, 1983). An essentially similar process probably occurrs at

the neuromuscular junction. An increase in calcium concentration also triggers catecholamine secretion from chromaffin cells of the adrenal medulla. Calmodulin binds with high affinity to chromaffin granule proteins and this promotes the binding of other cytosolic proteins (Geisow and Burgoyne, 1983). There is also evidence for the participation of calcium and calmodulin in exocytosis from nonneural cells. It is known that calcium participates both in platelet aggregation and in the release through exocytosis of substances which cause vasoconstriction and rapid clotting of the blood (Kaibuchi et al., 1983).

Renal handling of calcium

: Proximal convoluted tubule

Lassiter and co-workers (1963) firstly showed that the calcium concentration along the proximal convoluted tubule remained similar to that in the glomerulus, indicating the reabsorption of 59-60 percent of the filtered calcium along this segment. Calcium absorption in the proximal tubule may be active and transcellular or passive via the tight junctions. Passive calcium absorption could be secondary to fluid absorption, as a result of solvent drag, while passive diffusion could be secondary to a concentration gradient (lumen > peritubular space) (Brenner and Rector, 1986). Microperfusion techniques have been employed to investigate proximal calcium reabsorption. Frick and co-workers (1965) measured the efflux of calcium from perfusate

containing high calcium concentrations and the influx of calcium into calcium-free perfusate, and they observed striking similarities in the transport characteristics of calcium compared with those of sodium, suggesting the similar mechanisms of transtubule transport of these ions. Parathyroid hormone did not influence calcium reabsorption in the proximal tubule. Murayama and associates (1972) studied bidirectional fluxes of calcium in the perfused rat proximal tubule and observed that, as for sodium (Morel and Marayama, 1970), backflux into the tubular lumen was approximately three times the netflux, indicating that the proximal tubule is highly permeable to calcium.

: Loop of Henle

Rocha and colleages (1977) found in isolated perfused segments of rabbit nephron that the thin descending and ascending limbs of Henle's loop were relatively impermeable to calcium, the recent study of Bourdeau and Burg (1979) employing in vitro microperfusion of the isolated cortical thick ascending limb of the rabbit, confirms that there is an important component of passive net calcium flux could driven by the voltage in this segment. In more recent experiments, (Bourdeu and Burg, 1980) parathyroid hormone was shown to increase calcium reabsorption in the ascending limb, presumably by increasing calcium permeability. Suki and colleagues (1980) have reported that parathyroid hormone stimulates calcium reabsorption only in the cortical segment while calcitonin stimulates absorption only in the medullary segment of the thick ascending limb (Suki and Rouse, 1981).

: Distal convoluted tubule

Free-flow micropuncture studies have shown that 10-15 per cent filtered calcium is normally delivered to the superficial distal tubule, although less than 2 per cent reaches the final urine.

Costanzo and Windhager (1978) in free-flow micropuncture and droplet microinjection studies, confirmed that calcium is actively reabsorped along the superficial distal tubule and that this segment is highly impermeable to calcium, with insignificant backflux into the lumen. Futhermore, the high affinity Ca-Mg-ATPase (Doucet and Katz, 1982) is also present at its highest activity in these segments and may be responsible for active calcium extrusion from the epithelial cells. The distal tubule plays a major role in modulating urinary calcium excretion.

: Collecting duct

The collecting duct probably plays only a minor role in determining calcium excretion. A microcatheterization study of the inner medullary collecting duct demonstrated absorption of 1.4 per cent of the filtered load along this segment, which was apparently not affected by parathyroid hormone (Bengele et al., 1980).

Effects of hypercalcemia on cardiovascular functions

It is known that calcium may increase the contractility of both the heart (Shiner et al., 1969) and the peripheral blood vessels (Overbeck et al., 1961; Frohlich et al., 1962; Haddy et al.,1963; Marone et al., 1980). It is also observed in coronary vascular beds (Scott et al., 1961). In previous studies in man, acute hypercalcemia was reported to cause a slowing of heart rate (Shiner et al., 1969). Moreover, several lines of evidence suggest that the level of plasma calcium may plays a role in the regulation of blood pressure (Earll et al., 1966; Coburn et al., 1969; Rosenthall and Roy, 1972; Weidmann et al., 1972; Marone et al., 1980; Bianchetti et al., 1983), which show a significant correlation between the elevation in plasma calcium and the increase in blood pressure ($p<0.001$) (Weidmann et al., 1972; Marone et al., 1980; Bianchetti et al., 1983).

Effects of hypercalcemia on renal functions

The effects of hypercalcemia on renal functions are complex and have been studied extensively. A reduction in GFR has been noted in numberous studies (Ewards et al., 1974; Vanherweghem et al., 1976) and the mechanism was investigated by Humes and colleageues (1978). In the rat, infusion of calcium chloride was shown to result in a fall in both single-nephron and whole-kidney GFR, owing to a fall in capillary ultrafiltration coefficient (K_f) , but only in the presence of parathyroid hormone. Parathyroid hormone independently causes a

decrease in K_f and in GFR, (Ichikawa et al., 1978) and both receptors for parathyroid-sensitive adenyl cyclase (Imbert et al., 1974) have been demonstrated in glomeruli. It has been suggested that these changes in K_f could be secondary to changes in contractile state of mesangial cells, which could alter the glomerular capillary surface area (Humes et al., 1978).

Since calcium ions mediated in the vasoconstriction (Overbeck et al., 1961; Frohlich et al., 1962; Haddy et al., 1963) resulting to decrease both effective renal plasma flow and effective renal blood flow (Brian et al., 1974; Chomdej et al., 1977) and lead to decrease in the parallel of glomerular filtration rate in hypercalcemic states (Humes et al., 1978).

Several studies have indicated that hypercalcemia and hypocalcemia have direct effects upon tubule calcium transport. In the parathyroid intact animal, such effects may be mediated by parathyroid hormone (Edwards et al., 1974; Le Grimellec et al., 1974). However, even in the parathyroidectomized animal, calcium infusion increases absolute and fractional calcium excretion (Massry et al., 1968; Coburn et al., 1970). A fall in proximal fluid reabsorption in response to hypercalcemia has been observed in many studies (DiBona , 1971; Edwards et al., 1974) and natriuresis commonly occurs as a result of inhibition of tubule sodium reabsorption (Massry et al., 1968; Suki et al., 1969). However, calcium clearance is greater any level of sodium clearance than is observed with saline infusion, (Massry et al., 1968)

a finding consistent with selective inhibition of distal calcium transport, which in these studies may be related in part to parathyroid hormone suppression. In micropuncture experiment is the thyroparathyroidectomized dogs (Sutton et al., 1983) a rise in single-nephron filtered calcium load resulting from a rise in plasma calcium from subnormal to normal levels had an inhibitory effect on loop calcium reabsorption that was not observed when a similar change in filtered calcium load resulted from a change in GFR. These data suggested that plasma calcium level per se may influence tubule calcium reabsorption and this has been shown directly in the in vivo perfused loop of Henle of rat (Quamme, 1982) as well as in the in vitro perfused cortical thick ascending limb of the rabbit (Shareghi and Agus, 1982). These direct effects of the plasma calcium level on calcium transport in the thick ascending limb may be very important in determinating the final urinary calcium excreting in health and disease (Sutton , 1983).