CELLULAR PHARMACOLOGY OF LANTHANUM

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The general chemical characteristics of the rare earth or lanthanide series of elements resemble those of the alkaline earth elements in many respects (1). Of the lanthanides, lanthanum has long been considered to have chemical properties most similar to the alkaline earths (2). Recent interest in the biological actions of lanthanum ion (La^{3+}) is almost entirely based upon use of this rare earth ion as a substitute or antagonist for Ca^{2+} in a variety of cellular and subcellular reactions. The rapid and widespread employment of La^{3+} in the last few years is a measure of the complex and varied functions of Ca^{2+} in membrane and coupling reactions. In this review article, I attempt to concentrate upon the cellular actions of La^{3+} in muscle, nerve, and related tissues. A considerable literature has also accumulated concerning use of La^{3+} to discern the presence or absence of anatomical barriers and to delineate the molecular nature and specificity of subcellular Ca^{2+} transport systems and membrane structure. References to these areas of research (as well as to the general pharmacology of La^{3+}) will be limited to those studies most relevant to a consideration of the cellular basis of the actions of La^{3+}.

As long ago as 1910, Mines (3) examined the inhibitory effects of La^{3+} upon contractile function in frog heart. However, the recent proliferation of La^{3+}-related studies can quite clearly be traced to a prediction in 1964 by Lettvin and co-workers (4) that La^{3+}, by virtue of an ionic radius similar to Ca^{2+} and a higher valence than Ca^{2+}, will bind at superficially located Ca^{2+} sites in a less reversible manner than does Ca^{2+}. The subsequent testing of this prediction (5) demonstrated that La^{3+} did indeed exert a blocking action in lobster axons and that it acted in a manner equivalent to an extremely high Ca^{2+} concentration.

The high affinity of La^{3+} for membrane binding sites was of particular interest when related to evidence that the distribution of La^{3+} in many different preparations was confined to membrane areas contiguous with the extracellular space (6–12). Thus, use of La^{3+} as a relatively specific Ca^{2+} substitute or antagonist at well-defined

cellular locations appeared justified by both electrophysiological and anatomical criteria.

NERVE AND SKELETAL MUSCLE

Demonstration of blockade of Ca^{2+} fluxes by La^{3+} can be accomplished with least ambiguity in artificial membranes and in nerve cells. In a porous phospholipid-cholesterol artificial membrane, addition of 1 m*M* La^{3+} changed the membrane from a cation exchanger to an anion exchanger (13). This effect was interpreted as being due to the ability of La^{3+} to associate with fixed negative groups in a more pronounced manner than Ca^{2+}. More directly relevant for biological membranes is the demonstration by van Breemen & de Weer (14) that La^{3+} decreases the ^{45}Ca efflux rate by 87% when added to the solution bathing a squid giant axon previously injected with ^{45}Ca. The ^{45}Ca uptake in rabbit vagus nerve was also reduced by La^{3+} and the decrease was maintained (15). Thus, this effect of La^{3+} on Ca^{2+} uptake in vagus nerve can be interpreted as a blockade of at least a fraction of Ca^{2+} uptake rather than a decreased rate of uptake. In the same manner, La^{3+} irreversibly inhibited a lithium-sensitive Na^+ efflux component which was also abolished by removal of external Ca^{2+} (16). Again, the specific action of La^{3+} could be a Ca^{2+}-antagonistic action affecting the Ca^{2+} uptake component of a Na^+-Ca^{2+} exchange system.

Frog sartorius muscle is a tissue in which Ca^{2+} movements, contractile responses, and mechanisms of drug actions can be considered in terms of extensively characterized cellular structures (see 17, 18). The alteration by La^{3+} of specific ^{45}Ca movements in this muscle also can be readily described. In sartorius muscle, La^{3+} decreased ^{45}Ca uptake, transiently increased ^{45}Ca release, and blocked the increased ^{45}Ca uptake and tension response usually obtained with 80 m*M* K^+ but not with caffeine (19). Potassium ions act at least partially at sites in the transverse tubules (17, 20), whereas the effects of caffeine on sartorius contracture and ^{45}Ca efflux appear to be independent of the extracellular Ca^{2+} concentration (21, 22). Thus, in frog sartorius muscle, La^{3+} acts specifically at superficial sites (at cellular and transverse tubule membranes) to displace Ca^{2+} and to prevent tension responses and ionic movements associated with these sites (19). However, La^{3+} did not inhibit the caffeine-induced increase in ^{45}Ca uptake, an uptake which has been attributed to an increased cellular permeability to Ca^{2+} in both polarized and depolarized muscles (23). On this basis, it appears that not all Ca^{2+} uptake mechanisms in frog sartorius muscle are blocked by La^{3+}.

In frog sartorius muscle even contractile responses to high K^+ are not quantitatively related to the magnitude of the Ca^{2+} influx (24, 25) but, instead, the Ca^{2+} influx may act as a trigger for subsequent release of cellular Ca^{2+} (24). However, in some other muscle systems, Ca^{2+} entry is quantitatively important for both contractile responses and at least part of the depolarizing current.

Under these circumstances, the effects of La^{3+} on Ca^{2+} entry and subsequent events are quite pronounced. Of a number of divalent and trivalent ions tested, Hagiwara & Takahashi (26) found that La^{3+} was most potent in suppressing the

Ca-spike potential of the barnacle muscle fiber membrane. Binding of La^{3+} at the membrane surface appeared to be irreversible. In amphioxus muscle, La^{3+} also blocked the Ca^{2+}-dependent membrane conductance increases—presumably by occupying Ca^{2+} sites near or at the membrane (27).

In some situations involving either Ca^{2+}-dependent transmitter release mechanisms at the neuromuscular junction or slow twitch fibers (as in frog rectus abdominis muscle), La^{3+}-Ca^{2+} relationships appear more complex. In frog rectus abdominis muscle, La^{3+} inhibited ^{45}Ca uptake and the increase in residual ^{45}Ca content induced by high K^+ or acetylcholine (but not by nicotine); however, La^{3+} had only weak inhibitory effects on tension responses to high K^+, acetylcholine, or nicotine (28). Because removal of Ca^{2+} readily abolishes tension responses in rectus abdominis muscle, it is likely that responses dependent in some manner upon relatively superficial Ca^{2+} are resistant to inhibition by La^{3+}. Thus, even though La^{3+} is a relatively specific Ca^{2+} antagonist, it cannot be assumed that all responses dependent upon Ca^{2+} uptake or superficial binding of Ca^{2+} are susceptible to La^{3+} or even accessible to La^{3+}. More than one type of effect of La^{3+} on junctional transmission also has been described. A number of reports show that La^{3+} effectively increased spontaneous miniature endplate potential (MEPP) frequency (29–34) even in the absence of extracellular Ca^{2+} (31). At the same time, La^{3+} inhibited both the endplate (EP) potential (31) and the inward movement of Ca^{2+} necessary for normal transmitter release (35), whereas La^{3+} increased the rate of carbamylcholine-induced desensitization (36). Explanations offered for the potentiating effect of La^{3+} on spontaneous transmitter release (seen as an increase in MEPP frequency) include *(a)* accumulation of La^{3+} inside the nerve terminals and subsequent release of sequestered Ca^{2+} by this intracellular La^{3+} (31), and *(b)* an action of La^{3+} at superficial membrane sites to initiate release of Ca^{2+} from less superficial cellular stores (31, 33). Even though Heuser & Miledi (32) reported that La^{3+} causes structural changes in nerve terminals after longer incubation intervals (more than 1 hr), these changes paralleled a decline in spontaneous MEPP frequency and were not seen at shorter exposure intervals when increased spontaneous MEPP frequencies were observed. In the absence of evidence that significant amounts of La^{3+} enter the nerve terminal at a rapid rate, it seems more consistent with the actions of La^{3+} in other tissues to attribute the stimulatory action of La^{3+} to a postulated coupling between the binding of La^{3+} at external membrane sites and the release of sequestered Ca^{2+}.

CARDIAC MUSCLE

In cardiac muscle, the relationship between the development of contractile force and the level of extracellular calcium is a much more direct and obvious one than exists in fast twitch skeletal muscle. A recent review by Langer (37) provides a summary of heart muscle excitation-contraction coupling material and includes reference to some La^{3+} experiments in this area (38–40). Initial studies by Sanborn & Langer (38) on rabbit heart muscle demonstrated quite clearly that low concentrations of La^{3+} (5–40 μM) elicited both a transient increase in ^{45}Ca efflux and a decreased

tension response without significant alteration of the action potential. The La^{3+}-induced inhibition of tension was generally reversible if the exposure to La^{3+} was brief, and increased extracellular Ca^{2+} yielded a small and transient increase in the efflux of ^{140}La. The primary and specific action of La^{3+} in mammalian heart tissue thus appears to be an inhibition of Ca^{2+} uptake (and subsequent release) at superficial membrane sites (38, 41) from which Ca^{2+} release may not be directly related to induction of contraction but, rather, essential for the release of relevant Ca^{2+} from less superficial sites (41). The superficially located Ca^{2+} binding sites in cardiac muscle appear to be affected by both lack of oxygen and exposure to drugs. Naylor and co-workers have reported that the amount of Ca^{2+} displaced by La^{3+} from superficially located membrane sites is reduced in ischemic or hypoxic muscle (42) and by pentobarbital (43), whereas ouabain, in concentrations sufficient to give a positive inotropic response without contracture, increased the amount of Ca^{2+} displaced by La^{3+} (40). Thus, the superficial Ca^{2+} fraction affected by La^{3+} appears to be important for induced alterations in cardiac function.

Extrapolations of data obtained from hearts of only one mammalian species may not result in an accurate general picture. Dietrich & Diacono (44) employed La^{3+} in perfused hearts from rats and guinea pigs and found that ouabain-induced contractions were more directly dependent on Ca^{2+} influx in rat hearts, whereas in guinea pig hearts the amplitude and duration of depolarization (and, presumably, subsequent release of Ca^{2+} from membrane stores) was relatively more important. Cellular parameters affected by La^{3+} in heart cells can be described with more precision in heart cell cultures or embryonic heart cells. In chick embryonic hearts, the Ca^{2+} channels present during early development are blocked by La^{3+} (45). In cultured rat heart cells, La^{3+} prevents Ca^{2+} uptake, greatly reduces Ca^{2+} efflux, abolishes contractile tension but not the action potential, displaces Ca^{2+} at superficial membrane sites, and binds specifically to the basement membrane without penetrating beyond this region (39). Though cultured cells may differ in structural detail from intact adult heart cells, similarities in effects of La^{3+} indicate that contractile responses in most cardiac muscle systems are regulated by a superficially located and rapidly exchangeable Ca^{2+} component. The high degree of specificity with which La^{3+} interacts with these Ca^{2+} sites facilitates a dissociation of at least two functional Ca^{2+} compartments in cardiac muscle.

SMOOTH MUSCLE

Use of La^{3+} as a tool to block some but not all Ca^{2+} movements in various isolated smooth muscle preparations is particularly valuable because the morphological basis for storage and release of Ca^{2+} in this type of muscle cannot be described as clearly as has been done for other muscle systems. Attempts to obtain specific physiological and pharmacological alterations in Ca^{2+} movements and distribution in terms of ^{45}Ca fluxes in a variety of preparations including guinea pig ileal longitudinal smooth muscle (46, 47) and taenia coli (48–50), rat uterine smooth muscle (51–54), and rabbit aortic smooth muscle (55–57) have been hampered by the presence of quantitatively large and apparently unrelated ^{45}Ca movements. There appear to be

distinct differences between the manner in which contractile responses depend upon extracellular Ca^{2+} when responses to high K^+ are compared with those obtained with norepinephrine and histamine in rabbit aorta (56), norepinephrine in the rabbit ear vascular bed (58), epinephrine in rat ventral tail artery (59) and rat aorta (60, 61), and acetylcholine in rat uterus (62) and guinea pig taenia coli (63). In all of these preparations, removal of extracellular Ca^{2+} inhibits K^+-induced contractions more readily than contractile responses elicited with various stimulatory agents. Generally, this has led to a further inference concerning the nature of the Ca^{2+} important for contractile responses to K^+ and to other agents. Briefly, this hypothesis states that most of the Ca^{2+} important for K^+-induced smooth muscle contractions originates in the extracellular fluid or at superifical cellular sites or stores, whereas the major portion of the Ca^{2+} utilized by other stimulatory agents is located at less superficial or more sequestered sites or stores.

The presumption that smooth muscle contraction is linked to either depolarization by high K^+ or drug-receptor interactions by a number of intermediary steps that include Ca^{2+} movements or Ca^{2+}-mediated reactions has resulted in many investigations designed to elucidate the nature of these Ca^{2+}-dependent effects. This has been most conveniently accomplished by use of different types of inhibitory agents. Initial use of the local anesthetic cocaine in this manner led to the report by Hurwitz (64) that cocaine and Ca^{2+} exerted antagonistic actions on tension in K^+-depolarized longitudinal smooth muscle from guinea pig ileum. In polarized longitudinal smooth muscle, tone is inhibited by either high Ca^{2+} or cocaine (65). This indicates that interactions in depolarized smooth muscle are not the consequences of repolarization but, rather, are more closely related to events leading directly to smooth muscle contraction. The competitive nature of the inhibitory effects of local anesthetics upon Ca^{2+}-induced contractions in depolarized rat uterus has been described by Feinstein (66). Other inhibitory agents and the depolarized smooth muscle systems in which they exert a Ca^{2+}-antagonistic effect on contractile tone include ethanol in guinea pig ileal longitudinal smooth muscle (65), papaverine in guinea pig taenia coli (67), and phenoxybenzamine (68), desipramine (69), caffeine (70), cinnarizine, and chlorpromazine (71) in vascular smooth muscle preparations. However, K^+-depolarized preparations represent systems in which the patterns of Ca^{2+} binding and flux are substantially altered from the polarized state (72). Thus, effects of pharmacological agents on Ca^{2+} movements and contractile tone may also be qualitatively different in polarized and K^+-depolarized smooth muscles.

The effect of La^{3+} on membrane potential has not been measured directly in smooth muscle, though Anderson et al (73) found inhibition of both peak transient and steady-state currents in rat myometrical strips. However, if the role of La^{3+} is similar to that in nerve (5), a stabilizing or even hyperpolarizing action is likely. On this basis, Weiss & Goodman (74) predicted that La^{3+} would directly exert a stabilizing action at superficial Ca^{2+} sites but would only indirectly affect those Ca^{2+} sites or stores inaccessible to the extracellular bathing solution. The use of La^{3+} is then of particular value as a potential and specific antagonist of only a portion of total cellular Ca^{2+}. The idea that La^{3+} would, in essence, help dissociate Ca^{2+}-dependent actions has been successful in a number of important respects and has

increased understanding of drug-Ca^{2+} interactions in several different types of smooth muscle. Not surprisingly, the degree of dissociation between responses to high K^+ and other types of Ca^{2+}-dependent responses varies with the manner in which Ca^{2+} is taken up, stored, and utilized.

In ileal longitudinal smooth muscle, comparisons between effects of acetylcholine and those of high K^+ indicate that Ca^{2+} acting at superficial sites decreases membrane permeability to inorganic ions (65, 75). Depletion of Ca^{2+} (by washing out muscles in a calcium-free medium) rapidly abolishes responses to both high K^+ and acetylcholine (76). Furthermore, either high K^+ (77, 78) or acetylcholine (79) will increase smooth muscle tone in ileal longitudinal smooth muscles after incubation in a high Ca^{2+} solution and subsequent washout in a calcium-free medium. Under these conditions, Ca^{2+} mobilization from less superficial sites or stores may occur. The relationship between superficial Ca^{2+} and Ca^{2+} located at less superficial sites or stores in guinea pig ileal longitudinal smooth muscle has been studied in some detail by Hurwitz and co-workers (77–79). It appears that removal of superficial or stabilizing Ca^{2+} is linked to cellular Ca^{2+} release so that agents may initiate inward Ca^{2+} movements by removal of superficial Ca^{2+}. Conversely, inhibitory agents can prevent contractile responses in ileal longitudinal smooth muscle either by blocking loss of stabilizing Ca^{2+} or by displacing and replacing this Ca^{2+}. Thus, Weiss & Goodman (74) explained the effects of La^{3+} in this muscle by postulating that La^{3+} displaces Ca^{2+} from surface sites, binds well at these sites, and exerts a stabilizing action that prevents inward release of Ca^{2+} from less accessible membrane sites. Uptake of ^{45}Ca is also inhibited, and contractile responses to both acetylcholine and high K^+ are blocked. Further investigation by Goodman & Weiss (80) of the effects of lower La^{3+} concentrations on ileal longitudinal smooth muscle contractions elicited with high K^+ or acetylcholine indicates that no differential inhibitory effects were observed. Contractions in both cases were inhibited about 50% by a concentration of 0.9 μM La^{3+}. Thus, La^{3+} could not dissociate actions of agents affecting Ca^{2+} uptake from effects resulting from translocation of cellular Ca^{2+} in this smooth muscle system.

Use of La^{3+} to dissociate different cellular actions of stimulatory agents in other types of smooth muscle systems has been more successful. In rat uterine strips Goodman & Weiss (80) found that high K^+-induced contractions were inhibited by a 100-fold lower concentration of La^{3+} than were acetylcholine-induced contractions, and responsiveness to acetylcholine returned much more rapidly after exposure to La^{3+} than did that to high K^+. Similarly, in rabbit aortic smooth muscle, La^{3+} had a greater inhibitory effect on high K^+-induced contractions than on responses elicited with norepinephrine (81, 82). In aortic smooth muscle, La^{3+} inhibited the contractions obtained with histamine to a lesser degree than those by norepinephrine (82), whereas Ca^{2+} depletion (56) had the reverse effect (inhibition of histamine-induced contractions was greater than those elicited with norepinephrine). Lanthanum ion also irreversibly inhibited contractile responses to a number of agonists in the rabbit anterior mesenteric-portal vein (83) and blocked the tonic (high K^+-induced) response in guinea pig vas deferens (84). A recent postulation by van Breemen and co-workers (85) that histamine, norepinephrine, and angioten-

sin II may affect the same limited intracellular Ca^{2+} fraction in rabbit aorta was based upon the observation that, after La^{3+}, only one drug-induced contraction can be obtained with histamine, norepinephrine, or angiotensin II. These important drug interactions are complicated by the increased inhibitory activity of La^{3+} on smooth muscle contractile responses as the exposure interval is lengthened (G. B. Weiss and F. R. Goodman, unpublished observations). Further documentation of the time-dependent drug relationships involved would help clarify the manner in which these different agents may utilize similar Ca^{2+} stores. The uptake of ^{45}Ca is also blocked by La^{3+} in intestinal (74, 86), uterine (80), and vascular (82) smooth muscle under appropriate conditions.

An indirect approach to measurement of changes in cellular Ca^{2+} in smooth muscle has been proposed by van Breemen and co-workers (85, 87). Their idea, which they have termed "the Lanthanum method," is based upon the assumptions that a sufficiently high concentration of extracellular La^{3+} will *(a)* displace and replace extracellular Ca^{2+}, *(b)* block both Ca^{2+} uptake and efflux, and *(c)* not enter the cell in appreciable quantities to displace or alter cellular Ca^{2+} distribution. On this basis, tissues could be exposed to a variety of stimulatory agents or conditions in the presence of ^{45}Ca and subsequently placed into washout solutions containing a concentration of La^{3+} high enough to replace all extracellular or superficial Ca^{2+} and to prevent any further uptake or efflux of cellular ^{45}Ca. In this manner, effects on cellular ^{45}Ca uptake that have been obscured by much larger quantities of extracellular ^{45}Ca and by nonspecific ^{45}Ca movements can be detected. However, the method, as it has been used, is subject to some serious criticisms. First, Hodgson, Kidwai & Daniel (88) reported that ^{140}La entered the rat myometrial cell in significant quantities. Their evidence for this is based primarily on binding of La^{3+} to isolated subcellular components. There is no direct demonstration in any smooth muscle system that La^{3+} either enters or is excluded from the intracellular compartment of stimulated as well as nonstimulated cells. The most convincing experimental approach would employ autoradiographic or electron microscopic techniques. In the absence of this, the possibility cannot be excluded that La^{3+} may enter the cell in differing quantities (which may even relate to the prior treatment regime) and alter the pattern of ^{45}Ca washout in a differential manner.

Determination of the actual rate of ^{45}Ca washout during the period of exposure to La^{3+} is also essential. Van Breemen et al stated (89) that efflux of ^{45}Ca is blocked by La^{3+} in smooth muscle, but they demonstrated this only for smooth muscle treated with monoidoacetic acid and 2,4-dinitrophenol (89) and for other systems such as squid axon (85) and artificial phospholipid membranes (81). Actually, it is not essential that La^{3+} totally block ^{45}Ca efflux, but only that ^{45}Ca emerge at similar and constant rates from control and treated tissues during washout in the presence of La^{3+}. This should be ascertained for each type of preparation by measuring the rate of loss of ^{45}Ca during the period of exposure to La^{3+}. In the absence of evidence that desaturation-type washout curves are parallel, the possibility exists that small but significant variations in the rate of loss of ^{45}Ca during the period of exposure to La^{3+} may be in large part responsible for the differences observed in residual ^{45}Ca content. The concentration of La^{3+} also appears rather critical. Earlier experiments

of this type were performed with 2 mM La^{3+} (85, 87), but later work indicated that 10 mM La^{3+} might be a more satisfactory concentration (86, 89). In guinea pig ileal longitudinal muscle, Burton & Godfraind (90) reported that this La^{3+} concentration is sufficient to block all ^{45}Ca uptake and binding, because the resultant ^{45}Ca space of 0.35 ml/g equals the ^{14}C-inulin space. The use of such a high concentration of La^{3+} (10 mM) raises the possibility that nonspecific membrane stabilizing actions of La^{3+} may now be more prominent than specific Ca^{2+}-antagonistic actions. This high concentration of La^{3+} appears to inhibit the uptake of ^{14}C-sorbitol in this manner in metabolically depleted taenia coli (91). Hyperpolarization with La^{3+} in lobster axon membrane was reported by Takata and co-workers (5) to be about 20 times as effective as with equivalent concentrations of Ca^{2+}. If similar actions occur in smooth muscle, it is possible that polarization-induced changes with 10 mM La^{3+} could alter the binding and mobility of cellular Ca^{2+}. Thus, increased cellular residual ^{45}Ca levels might be more a function of nonspecific stabilizing actions of La^{3+} rather than of specific Ca^{2+}-antagonistic effects.

OTHER SYSTEMS

The relationship between Ca^{2+} and many secretory processes has been extensively studied, and this area is summarized in a recent review by Rubin (92). The analogy between excitation-contraction coupling in muscle and stimulus-secretion coupling is firmly based upon the necessity for Ca^{2+} as a coupling agent in both types of processes. Thus, in view of the successful use of La^{3+} as a specific Ca^{2+} antagonist in muscle and nerve, it is not surprising that similar approaches with La^{3+} would be attempted in various secretory systems.

It might be expected that physiological similarities between hormonal release mechanisms and myoneural junction transmission would result in analogous stimulatory and inhibitory effects of La^{3+}. The Ca^{2+}-dependent secretory actions that La^{3+} has been reported to alter include catecholamine release from adrenal medulla (93) and histamine release from mast cells (94). In isolated bovine adrenals, Borowicz (93) found that only the first exposure to La^{3+} stimulated release of a large quantity of catecholamine and, conversely, La^{3+} inhibited the increased catecholamine release obtained with acetylcholine or high K^+. In a similar approach, Foreman & Mongar (94) report that La^{3+} can increase the spontaneous release of histamine but is a potent inhibitor of the calcium-dependent component of antigen-stimulated histamine release. The stimulatory actions of La^{3+} on spontaneous release of stimulatory agents, and the inhibitory actions of La^{3+} on induced release of stimulatory agents obviously parallel similar actions of La^{3+} (29–35) on spontaneous MEPP frequency and on end plate potential and Ca^{2+} uptake in junctional transmission. In neither of these two studies (93, 94) involving secretory actions was ^{45}Ca movement examined, but in both cases the La^{3+}-induced inhibition of Ca^{2+}-dependent stimulated release was attributed to a specific block by La^{3+} of Ca^{2+} uptake. Perhaps the stimulatory action of La^{3+} is also similar in mechanism to that observed in junctional transmission and may result from a coupling action between the binding of La^{3+} at superficial membrane sites and the release of sequestered Ca^{2+}. Another study involving effects of La^{3+} on a secretory system (rat mammary

tissue) was performed by Lawson & Schmidt (95). The oxytocin-induced milk ejection response of the tissue was markedly reduced by La^{3+}, and this action was attributed to a displacement by La^{3+} of superficial Ca^{2+} important for contraction of the myoepithelial cell. Unfortunately, as the authors noted, examination for possible displacement of superficially bound ^{45}Ca by La^{3+} was not undertaken in the absence of prior exposure to extracellular Ca^{2+}, and the resultant lack of effect is not conclusive. Similarly, failure to observe an oxytocin-induced increase in ^{45}Ca influx might be due to use of too low a concentration of La^{3+} (1 m*M*) to achieve substantial inhibition of ^{45}Ca efflux during the subsequent washout.

Employment of La^{3+} as a tool to examine Ca^{2+}-dependent actions has been attempted in numerous biological systems under various conditions. Though it is not feasible to discuss all of these studies at this time, some of the more interesting ones include (*a*) a reduction by La^{3+} in the increased frequency of ciliary beating, which results from mechanical stimulation and is related to the extracellular Ca^{2+} concentration and, presumably, to the Ca^{2+} influx which follows (96), (*b*) a removal by La^{3+} of large quantities of extracellular ^{45}Ca during washout of rat cerebral cortex slices (97), (*c*) an increase in membrane potential stability of Ehrlich ascites tumor cells in La^{3+}-containing solutions (98), (*d*) an enhancement by La^{3+} of in vitro calcification of the excised tibial epiphyses of Ca^{2+}-deficient (rachitic) rats (99), and (*e*) substitution of La^{3+} for Ca^{2+} essential for the postulated formation of an amiloride-receptor complex that blocks access of Na^+ to frog skin transport channels (100).

The effects of La^{3+} on Ca^{2+} uptake important for the binding and transport of Ca^{2+} in various subcellular structures and proteins has been the focal point for a number of studies concerned with the relevant biochemical and molecular mechanisms. Though a detailed discussion of these reports is outside the scope of this review, a summary of some basic views and concepts about binding of Ca^{2+} and La^{3+} is of considerable value. There is no doubt that La^{3+}, in extremely low concentrations, inhibits the uptake or binding of Ca^{2+} in rat liver mitochondria (101, 102) and in cardiac muscle sarcoplasmic reticulum (103, 104), but apparently not in dog cardiac microsomes (105). Of particular interest is the finding that specific high and low affinity sites for the binding of Ca^{2+} may exist in a protein fraction solubilized from rat skeletal muscle sarcoplasmic reticulum (106) and in rabbit skeletal muscle sarcoplasmic reticulum (103). Furthermore, La^{3+} is a relatively poor inhibitor of Ca^{2+} binding at specific Ca^{2+} sites in rabbit sarcoplasmic reticulum (103), and La^{3+} can also bind, in large amounts, to sites that differ from those normally occupied by Ca^{2+} (102). Thus, the suggestion emerges from these studies that the affinities of La^{3+} and Ca^{2+} for different membrane sites may be quite variable. The basis for differing affinities may be a function of access to sites, steric configurations in the vicinity of the sites, or other factors. Regardless of the molecular basis for these variations, the implication to be derived for isolated tissue studies is that, in a given cellular system, it cannot be assumed that La^{3+} has a greater affinity than Ca^{2+} for all relevant binding sites. The existence of specific La^{3+}-insensitive Ca^{2+} binding sites or uptake mechanisms may account for lack of inhibition by La^{3+} of the increased ^{45}Ca uptake elicited by caffeine in frog sartorius muscle (19) or by nicotine in frog rectus abdominis muscle (28).

GENERAL CONSIDERATIONS

The major point which emerges from consideration of the cellular pharmacology of La^{3+} is that this ion is a specific antagonist of Ca^{2+} in biological systems. It is possible to explain all of the effects of reasonable concentrations of La^{3+} by postulating that La^{3+} can replace Ca^{2+} at well-defined tissue loci or sites and, in this manner, either impede or augment Ca^{2+}-dependent movements or reactions. Use of La^{3+} to elucidate Ca^{2+}-dependent mechanisms of action appears to provide a more precise approach than use of a procedure such as Ca^{2+} depletion, whether accomplished by ionic variation or by addition of Ca^{2+}-chelating agents. Thus, the experimental value of La^{3+} has received rapid recognition, and La^{3+} has been used in a variety of investigations of Ca^{2+}-dependent processes over the last few years. Even with the increased current knowledge of La^{3+}-Ca^{2+} interactions, a number of significant problems remain. In each isolated system employed, dose-response relationships for La^{3+} as well as for Ca^{2+} should be ascertained routinely to prevent erroneous comparisons and extrapolations. This is particularly true if conclusions are to be based upon assumptions that La^{3+} blocks all Ca^{2+} movements or displaces virtually all Ca^{2+} from particular binding sites. It is clear that different types of preparations vary considerably in their sensitivity to inhibitory effects of La^{3+}. It is also possible that the relative affinities of Ca^{2+} and La^{3+} for different membrane binding sites may not be the same. Furthermore, high concentrations of La^{3+} may well exert nonspecific (e.g. stabilizing) effects or even actions that are deleterious to cellular integrity. More complete understanding of these potentially toxic actions of La^{3+} would be valuable. Related to this is the question of possible cellular penetration of La^{3+} under physiological conditions. Resolution of this problem, preferably by use of established electron microscopic techniques in each type of isolated tissue system, is essential for validation of much of the work based on use of La^{3+} to dissociate different cellular Ca^{2+} sites or stores.

Finally, it should be recognized that even though use of techniques involving La^{3+} will contribute greatly to resolution of Ca^{2+}-dependent actions, development of other tools and approaches also will be necessary. For example, an agent or agents that would specifically displace Ca^{2+} from sequestered cellular stores (particularly in smooth

with actions of Ca^{2+} at clearly defined cellular sites. In this manner, employment of La^{3+} as a partial and specific Ca^{2+} antagonist may well serve as a model for eventual use of additional agents in similarly defined roles in the elucidation of other Ca^{2+}-dependent biological actions.

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