EDITORIAL REVIEW

Calcium antagonists and hormone release

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Introduction

Calcium (Ca²⁺) has been aptly entitled the 'universal provocateur' [1] in recognition of its many indispensable metabolic actions. The class of drugs popularly known as calcium antagonists (calcium slow channel blockers) provokes a variety of pharmacological effects and these drugs are useful as probes of calcium-dependent mechanisms. One area where such application appears particularly appropriate is the study of hormone release, since it has been demonstrated that secretion of at least some hormones is dependent on translocation of extracellular Ca²⁺ across the cell membrane. Furthermore, specific physiological stimuli to secretion of most hormones can be applied in vivo or mimicked in vitro, and hormone levels may be measured by specific assays. Calcium antagonists also provide a possible means of studying the role of Ca²⁺ in hormone release in the intact organism. In many studies an inhibitory effect of a calcium antagonist on hormonal responses to secretory stimuli has been taken as evidence that transmembrane flux of Ca²⁺ is an obligatory event in the response. Such a conclusion begs several questions, which will be discussed in this review. For example, it is important to define the extent to which calcium antagonists represent a homogeneous class of drugs, whether 'antagonism' is restricted to inhibition of inward calcium flux, and whether the pharmacological actions vary according to the agent used or the cell type under study. Answers to such questions are necessary to exclude actions of calcium antagonists other than prevention of calcium uptake which may inhibit hormone release.

Calcium and hormone release

The role of Ca²⁺ in hormone release was first demonstrated by Douglas and his colleagues, who showed that extracellular calcium was necessary for release of adrenal medullary catecholamines and of vasopressin (AVP) from the neurohypophysis [2, 3]. These tissues were chosen because of their embryological and structural similarity to neuronal tissue, for which calcium-dependency of neurotransmitter release had previously been demonstrated [4]. The term 'stimulus-secretion coupling' was coined to describe a process in which the inward calcium flux is an inevitable concomitant of the stimulus and a necessary and sufficient determinant of the hormonal response, by analogy with the electro-mechanical ('excitation-contraction') coupling characteristic of muscle.

It has become apparent that owing to differences in the mechanisms of synthesis, intracellular handling, and release of hormones and in the role of Ca²⁺, stimulus-secretion coupling as originally defined is not a universal process. For example, the above hormones are secreted by exocytosis of previously formed storage granules and the secretion rate during acute stimulation is unaffected by variations in the rate of synthesis; the role of calcium is seen as facilitation of the exocytotic process. However, many hormones exhibit calcium-dependent release mechanisms but a secretion rate which is determined largely by synthesis. Secretion rates are also related in many cases to occupancy of specific receptors by a secretagogue and responses may be mediated by stimulation of adenylate cyclase and generation of cyclic adenosine 3':5'-phosphate (cAMP), in addition to calcium-dependent processes. Calcium channels for receptor-mediated events are thought to differ from those involved during depolarization [5]. In many cases it is uncertain whether: (i) calcium acts at a single locus; (ii) calcium affects synthesis or release; (iii) transmembrane calcium flux is an essential part of the secretory process; (iv) calcium and adenylate cyclase act independently or conjointly. These considerations have been discussed by Rasmussen [6], who pro-
posed the term ‘stimulus-response coupling’ to denote any specific cellular response to a physiological stimulus which is wholly or partly determined by calcium. This term is preferred since no particular mechanism is implied. The role of calcium in secretory processes has been comprehensively reviewed by Rubin [7].

It can be said then, that the role of calcium in hormone release is both variable and complex and may not depend solely on inward transmembrane flux.

**Pharmacology of calcium antagonists**

Calcium antagonists are a group of structurally dissimilar compounds which are defined pharmacologically by their ability to inhibit, in parallel and stereospecifically, the plateau phase of the action potential and agonist-induced contractions in various cardiac and smooth muscle preparations [8-10]. Measurements made with voltage clamp techniques suggest that inhibition of calcium influx also occurs [11], and hence blockade of the slow calcium channels is regarded as the prime mechanism of action of these drugs. In mammalian tissues, the rapid tetrodotoxin-sensitive phase of the action potential, which depends on ‘fast’ influx of Na+, is affected only at high concentrations [11, 12]. Other compounds such as transition metal cations (Mn²⁺, Co²⁺, La²⁺), local anaesthetics, phenytoin and phenothiazines, also affect calcium binding but are not normally classified as calcium antagonists. However, some overlap in properties exists, especially with the so-called calmodulin inhibitors such as trifluoperazine.

Several lines of evidence confirm that in myocardium and smooth muscle the main effect of calcium antagonists is blockade of calcium uptake. The effects of calcium antagonists are mimicked by decreasing extracellular [Ca²⁺] and decreased competitively when [Ca²⁺] is increased [13]. When myocardial fibres are ‘skinned’ by detergents (so removing the sarcolemma), calcium antagonists do not affect contractions stimulated by direct application of calcium and adenosine triphosphate (ATP) [14]. Calcium content, influx and egress from isolated subcellular particles such as sarcoplasmic reticulum and mitochondria are not affected by calcium antagonists [15, 16], except at high concentrations [17]. Skeletal muscle, in which contraction depends predominantly on mobilization of intracellular calcium, is little affected by calcium antagonists, though muscle cramp has been reported as a side-effect [18]. The correlation between inhibition of slow channel calcium influx and the concurrent decrease in contractile force [19] is further evidence for the above mechanism of action.

In smooth muscle and myocardium, mechanical responses depend on recruitment and ‘activation’ of stored intracellular calcium, as well as on calcium influx. Thus abolition of responses by calcium antagonists may be due to intracellular actions in which these processes are inhibited. Such actions have been suggested for nifedipine [20] and verapamil [21], though it is likely that they are additional to membrane effects and occur at higher concentrations [21]. In general, evidence for complementary actions of calcium antagonists is indirect. Individual drugs exhibit selective effects on different tissues, indicating subtle differences in pharmacology. For example, nifedipine is a potent peripheral and coronary vasodilator whereas verapamil has less marked vascular effects but inhibits intracardiac conduction, especially at the atrioventricular node. Verapamil inhibits phosphodiesterase [22] and exhibits membrane-stabilizing properties [23], which may contribute to its effect in cardiac and other tissue. Specific binding of [³H]nifedipine and [³H]nitrendipine to plasma membrane binding sites is only weakly displaced by verapamil, D600 (methoxyverapamil) and diltiazem [24, 25], suggesting different loci of action of the various structural subtypes. Vasoconstriction in dog mesenteric arteries in vitro in response to K+ and noradrenaline is abolished by nifedipine even when a calcium-free medium containing EGTA [ethanedioxybis(ethy1amine)tetra-acetic acid] is used [26]. Direct measurements using ⁴⁵Ca have failed to demonstrate inhibition of calcium uptake by verapamil, diltiazem, nifedipine and felodipine [20, 27], except in lanthanum-treated tissue [28]. It was suggested that felodipine acts by an interaction with calmodulin rather than by prevention of calcium uptake [27]. The difficulty may be due in part to the concurrent increase in calcium efflux which occurs during muscle contraction [29, 30] and also during stimulation of hormone release in several endocrine tissues [31-33]. It is of interest that calcium antagonists inhibit calcium efflux from erythrocytes in a dose-dependent manner [34] and also from myocardial cells [35].

It should be noted that the electrophysiological techniques commonly employed in the study of calcium antagonists do not provide information on calcium flux not associated with changes in membrane potential or on changes in these movements induced by drugs. Such processes include intracellular translocation of calcium, entry or egress of calcium which occurs by isoelectric exchange with other cations, or egress of calcium occurring during repolarization (since the
bulk of the current is carried by K⁺). Electrophysiological techniques are also inappropriate to the study of tissues which exhibit stimulus-response coupling but little or no change in membrane potential, such as some endocrine tissues.

Information is required on whether calcium antagonists actually enter cells. The volume of distribution of verapamil after intravenous administration is high (approx. 51/kg), suggesting significant tissue penetration [36]. However, this is not the case with tiapamil [37]. Godfraind demonstrated uptake of [³H]flunarizine into isolated rat aorta, but this occurred at saturable binding sites on the plasma membrane, and no radioactivity was found in nuclear or mitochondrial fractions [38]. This finding militates against an intracellular mechanism of action and is consistent with the observation that direct injection of D600 into perfused barnacle muscle fibre has no effect on inward calcium current or contractile responses [39].

Finally, individual tissue responses to calcium antagonists may vary according to the precise site examined. This is clearly seen in vascular smooth muscle in which membrane ATP-ase-driven ion pumps [40] and the effect of calcium antagonists [41] vary with vessel diameter.

The above considerations suggest that the assumption of a unimodal and universal mechanism of action for calcium antagonists is unwarranted, and that inhibition of responses cannot be interpreted with certainty.

Hormone release from individual endocrine tissues and the effect of calcium antagonists

Posterior pituitary (neurohypophysis). Neurohypophysal cells are capable of a depolarization similar to that in neuronal tissue [42], and exocytosis of AVP and oxytocin, along with the neurophysins, is critically dependent on changes in transmembrane calcium flux [2, 31]. D600 blocks release of AVP and oxytocin in vitro, and the inhibition is reversed by addition of calcium to the incubation fluid [43]. These results are consistent with the orthodoxy view of stimulus-response coupling and its prevention by calcium antagonists via inhibition of calcium uptake. It would be of interest to determine whether release of these hormones in response to acute stimuli in vivo, for example cigarette smoking, is prevented by calcium antagonists.

Verapamil also inhibits target organ hydro-osmotic responses to 'antidiuretic hormone' by modulation of a calcium-adenylate cyclase interaction [22].

Anterior pituitary (adenohypophysis). Action potentials may be generated in anterior pituitary cells by depolarizing currents or hormonal stimulation [44]. In addition, growth hormone (GH), gonadotropins (LH and FSH), adrenocorticotropin (ACTH) and thyrotropin (TSH), but not prolactin (PRL), are released by excess K⁺ in vitro [45, 46]. Calcium is required for hormone secretion induced by both K⁺ and hypothalamic releasing factors [47], but the effects of each stimulus are additive, suggesting that more than one calcium-dependent pathway is present [48]. Inhibition of TSH release by tri-iodothyronine is also calcium-dependent [33]. Secretion of PRL by cloned pituitary cells is sensitive to calcium [45, 49] and inhibited by verapamil [50]. Inward movement of Na⁺ and Ca²⁺ occurs in anterior pituitary cells during stimulation, and verapamil and D600 block ⁴⁵Ca influx [51]. In man, verapamil inhibits release of LH, FSH and TSH (but not PRL) in response to trophic stimulation [52]. The inhibition affects both the immediate and the late phases, due to release of pre-formed and newly synthesized hormone respectively [53]. Basal FSH and LH levels were also decreased by verapamil [52]. In rat pituitary tumour cells in vitro, trifluoperazine blocks Ca²⁺-dependent action potentials and inhibits GH and PRL release [54]. This finding suggests an interaction between calmodulin and pituitary hormone release.

Recently we have repeated the protocol of Barbarino & de Marinis [52] using nifedipine. No reduction in basal or stimulated FSH, LH, TSH or PRL levels was found [55]. These findings emphasize the heterogeneity of calcium antagonists and cast doubt on the critical nature of Ca²⁺ influx in anterior pituitary hormone release in vivo [56].

Thyroid. Thyroid follicular cells do not contain secretory granules. Calcium participates in the regulation of synthesis and possibly secretion of iodine-containing hormones but the mechanisms are unclear [57]. A role for redistribution of intracellular calcium has been proposed [58] but it is more likely that the effects of TSH are mediated via stimulation of adenylate cyclase [59]. The effect of calcium antagonists on stimulation of thyroxine by TSH has not been measured, but in our studies with nifedipine no effect of the drug on serum thyroxine was apparent [55].

Endocrine pancreas. The mediation of insulin and glucagon release by calcium has received wide attention. Insulin is stored in secretory granules and secreted by exocytosis, and D600-suppressible changes in membrane potential and ⁴⁵Ca²⁺ influx and efflux have been noted during stimulation of insulin release [60, 32]. Thus islet tissue exhibits some of the properties already described for the
neurohypophysis. However, the effect of Ca²⁺ antagonists on insulin release has been controversial. Studies in vitro have consistently shown inhibition of insulin release by nifedipine, diltiazem, verapamil and lanthanum [61-65]. Verapamil exhibits a long-term inhibitory effect on synthesis of pro-insulin, in addition to acute inhibition of insulin release by nifedipine, diltiazem, and lanthanum [61-65]. Verapamil and La³⁺ inhibit aldosterone responses to both secretagogues in rat zona glomerulosa cells in vitro [86] and in man nifedipine diminishes the responses to infused ANG II when given acutely [87, 88] but not chronically [89, 90]. However, during chronic nifedipine treatment aldosterone levels are less than those expected from concurrent values for circulating renin [91]. Transmembrane ⁴⁵Ca flux in superfused zona glomerulosa cells during acute ANG II stimulation shows a net efflux [92]. Since calcium antagonists inhibit efflux of ⁴⁵Ca²⁺ in erythrocytes [34], it is possible that the action of verapamil and nifedipine on aldosterone release results from blockade of Ca²⁺ egress rather than inhibition of uptake.

**Parathyroid and thyroid 'C' cells.** Parathyroid hormone (PTH) and calcitonin form a unique system subserving calcium homeostasis, and the relationship between extracellular (plasma [Ca²⁺]) and hormone secretion is exceptional. Both hormones are secreted in proportion to [Ca²⁺] but the correlation is negative for PTH [93]. PTH secretion occurs in the absence of extracellular calcium and transmembrane calcium influx [94]. The reciprocal responses of PTH and calcitonin to changes in extracellular calcium probably derive from opposite effects on the respective rates of synthesis [95]. The effect of calcium antagonists on PTH or calcitonin secretion in normal or pathological states has not been studied, but in our studies free [Ca²⁺] was not changed after nifedipine [55].

**Testis and ovary.** Omission of calcium from the incubation medium of Leydig cells in culture decreases the testosterone responses to dibutryl-cAMP and to high doses of chorionic gonadotropin [96]. Testosterone production but not cAMP-dependent protein kinase activity in response to LH was similarly decreased [97], suggesting that calcium acts after stimulation of adenylate cyclase. Lin et al. noted inhibition of LH- and dibutyryl-CAMP-stimulated interstitial cell steroidogenesis by verapamil in vitro [98]. The concentration-dependence of the inhibition again suggested an action beyond cAMP formation, and pointed to an intracellular locus of drug action. These studies therefore provide evidence for effects of calcium antagonists on hormone release not mediated by prevention of calcium uptake.

The effects of calcium antagonists on ovarian hormone secretion, or on the secretion of inhibin from the testis [99], have not been studied.

**Kidney (juxtaglomerular apparatus).** Infusion of calcium into the renin
release [100], and inhibition of renin release by ANG II is calcium-dependent [101]. The relationship, if any, between calcium and release of inactive renin (prorenin) is not known. Inhibition of active renin release by ANG II is not affected by D600 in vitro [102] or nifedipine in vivo [103] in spite of the dependence on extracellular calcium. However, diltiazem and verapamil antagonize the inhibitory effects of K⁺ and AVP respectively on renin release in vitro [104, 105]. Since calcium antagonists increase renal blood flow [106], any direct effect on renin release in vivo may be obscured by secondary changes. In our experience nifedipine stimulates renin release acutely [103] but renin levels after chronic dosing with nifedipine or verapamil are unchanged [107, 108]. Circulating inactive renin levels are unchanged by nifedipine [103].

These studies confirm that the role of calcium in renin release is complex and suggest that calcium antagonists are not suitable for whole animal studies of renin release in spite of measurable effects in vitro.

Conclusions

Calcium antagonists are useful in the study of hormone release mechanisms, but it should be remembered that they are a heterogeneous group of drugs which probably affect cell calcium in several ways other than prevention of calcium influx. Hormone release is likewise not a uniform process, and the role of calcium in hormonal responses varies. Therefore, inhibition of hormone responses by calcium antagonists is not sufficient proof of a critical requirement for uptake of calcium in hormone release. Negative findings in whole animals may be due to secondary mechanisms or efficient homeostasis. We suggest that caution is required in the use of calcium antagonists to study mechanisms of hormone release in vivo.

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References


