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CALCIUM CHANNELS AND THEIR INVOLVEMENT IN CARDIOVASCULAR DISEASE

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Abstract—The widespread distribution of L-type Ca^{2+} channels in the cardiovascular system makes that system a natural 'target' for drugs which inhibit L-type Ca^{2+} channel activity. Now that tissue-dependent differences in the chemical composition of the calcium antagonist binding sites have been recognized it may be possible to develop drugs with enhanced tissue selectivity. The search for new compounds should not be restricted to improvements in tissue selectivity, however. Some of the ancillary properties of the L-type Ca^{2+} channel inhibitors—including their ability to protect against lipid peroxidation—should not be lost because these ancillary properties may contribute significantly to their usefulness as therapeutic agents.

Ion-conducting channels which normally admit extracellular Ca^{2+} and which are voltage sensitive and relatively selective for Ca^{2+} are present in most cells [1]. Based on their pharmacological and physiological profiles it has been customary to subclassify these channels into L, T or N subtypes [2] but with the benefit of hindsight this classification may be too restrictive. For example, it is now known that dendrites of cerebellar Purkinje cells contain voltage-sensitive, Ca^{2+}-selective channels, the pharmacological and biophysical properties of which prevent them from being classified in this way. Currently these Ca^{2+} channels are known as P-type channels [3] and one of their distinguishing peculiarities is that, unlike the classical L, T, or N type Ca^{2+} channels, they are sensitive to the toxin of the funnel-web spider [4]. There are probably other types of Ca^{2+} channel but this paper is concerned primarily with the L-type Ca^{2+} channel, since it is this type of Ca^{2+} channel which abounds in the cardiovascular system.

L-type Ca^{2+} channels are easily identified in terms of their relatively large capacitance, high threshold of activation and sensitivity to a wide variety of organic and inorganic antagonists, including (Table 1) the recently identified polypeptide calciseptine [5]. This particular polypeptide contains 60 amino acids, eight of which are cysteine residues (Table 2). It is present in the venom of the black mamba snake and is the only known naturally occurring polypeptide which specifically antagonizes L-type Ca^{2+} channel activity.

Within the last few years the L-type Ca^{2+} channel has been isolated and cloned. Its subunits have been identified and its amino acid composition—including some tissue-dependent peculiarities—determined [6]. In addition abnormalities in Ca^{2+} channel structure and function have been identified as being associated with certain naturally occurring and drug-induced pathological conditions [7].

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The structure of the Ca^{2+} *antagonist L-type* Ca^{2+} *channel*

The mammalian voltage-dependent, calcium antagonist-sensitive Ca^{2+} channel is an oligomeric structure consisting of five putative subunits—designated α_1, α_2, β, γ and δ [8]. Figure 1 illustrates its probable method of assembly [8]. Of these subunits it is the α_1 subunit which almost certainly constitutes the ion-conducting component of the complex because, when incorporated into planar lipid bilayers [8–11] or microinjected into cells which lack an endogenous α_1 subunit [12], it functions as a calcium-antagonist sensitive, Ca^{2+}-conducting channel. This α_1 component of the channel also contains the voltage sensor for the channel [13].

The α_1 subunit of the channel is not extensively glycosylated and it is strongly hydrophobic. Its precise amino acid composition shows some tissue specificity. For example, in cardiac and aortic smooth muscle cells there is a close similarity in composition (93% homology, Table 3), indicating that the aortic smooth muscle α_1 subunit closely resembles its cardiac muscle equivalent (Table 3). However, the aortic, cardiac and brain α_1 subunits are only between 65 and 75% homologous with their skeletal muscle equivalents [6, 14].

Irrespective of whether the α_1 subunit belongs to a Ca^{2+} channel complex associated with cardiac, vascular smooth muscle or even skeletal muscle, the amino acids which constitute the subunit are arranged in four repeating hydrophobic motifs each of which contains six membrane spanning segments (Fig. 2). One of the most remarkable features of this arrangement—apart from its homology with the Na^+ channel [15]—is that segment S4 of each motif appears to be highly conserved in terms of its amino acid composition, in particular with respect to the presence of an arginine or lysine residue at the third position.

It is beyond the scope of this paper to discuss either the biochemistry or the functional significance of the other subunits (α_2, γ, β, and δ) of the Ca^{2+}

Table 1. Characteristics of voltage-dependent L-, T- and N-type calcium channels

Property	Channel Type L	T	N
Conductance (ps)	25	8	12–20
Activation threshold	High	Low	High
Inactivation rate	Slow	Fast	Moderate
Sensitivity to calcium antagonists: phenylalkylamines, benzothiazepines, dihydropyridines	Sensitive	Insensitive	Insensitive
Sensitivity to calciseptine*	Sensitive	Insensitive	Insensitive

* Calciseptine, a polypeptide, is a naturally occurring calcium antagonist (see Table 2).

Table 2. Chemical composition of calciseptine

Amino Acid	Key	Amino Acid	Key
Arginine	R	Arginine	R
Isoleucine	I	Glutamic acid	E
Cysteine	C	Tyrosine	Y
Tyrosine	Y	Isoleucine	I
Isoleucine	I	Serine	S
Histidine	H	Glutamic acid	E
Lysine	K	Arginine	R
Alanine	A	Glycine	G
Serine	S	Cysteine	C
^{10}Leucine	L	^{40}Glycine	G
Proline	P	Cysteine	C
Arginine	R	Proline	P
Alanine	A	Threonine	T
Threonine	T	Alanine	A
Lysine	K	Methionine	M
Threonine	T	Tryptophan	W
Cysteine	C	Proline	P
Valine	V	Tyrosine	Y
Glutamic acid	E	Glutamine	Q
^{20}Asparagine	N	^{50}Threonine	T
Threonine	T	Glutamic acid	E
Cysteine	C	Cysteine	C
Tyrosine	Y	Cysteine	C
Lysine	K	Lysine	K
Methionine	M	Glycine	G
Phenylalanine	F	Aspartic acid	D
Isoleucine	I	Arginine	R
Arginine	R	Cysteine	C
Threonine	T	Asparagine	N
^{30}Glutamine	Q	^{60}Lysine	K

Table 3. Tissue specificity of the α_1 subunit

Origin	% Homology relative to Cardiac α_1	Skeletal α_1
Cardiac muscle	—	65
Aortic smooth muscle	93	66
Brain	—	75
Skeletal	65	—

channel complex, other than pointing out that they probably provide sites for glycosylation and cyclic AMP- and GMP-dependent regulation, as well as stabilizing the Ca^{2+} channel complex in the membrane. Of relevance to the present paper, however, is the tissue specificity of the α_1 subunit.

Tissue specificity of the α_1 subunit

Arguments favouring the hypothesis of tissue specificity with respect to this subunit include the following:

(a) mice born with the genetically determined and fatal condition of muscular dysgenesis lack functional α_1 receptors in their skeletal but not cardiac Ca^{2+} channel complexes [16] which is indicative of different gene coding for the two tissues.
(b) mRNA blot analysis with probes for the skeletal muscle α_1 subunit show the expected strong band for skeletal muscle, only a weak hybridizing signal for cardiac muscle DNA and no signal for ileal or brain tissue [17].
(c) Injecting cDNA plasmids coding for cardiac α_1 subunits into skeletal muscle myotubules obtained from dysgenic mice which lack functional α_1 subunits results in the restoration of α_1 subunit activity as indicated by the restoration of Ca^{2+} current activity but the Ca^{2+} currents are now of the cardiac type, and the tissue is dependent on the presence of extracellular Ca^{2+} for excitation–contraction coupling [16].
(d) The amino acid composition of the terminal intracellular carboxy tail (Fig. 2) of the aortic α_1 subunit is different from that obtained for cardiac (or skeletal) muscle [6].

In general, therefore, although the α_1 subunit of the Ca^{2+} channel complex is well preserved in brain, cardiac, vascular smooth and skeletal muscle; there are subtle differences in the subunit between the different muscle types, including differences in amino acid composition and the length of the carboxy terminus [6]. These differences may contribute to the tissue specificity which some of the prototype calcium antagonists display because it is the α_1 subunit which selectively binds these drugs [18].

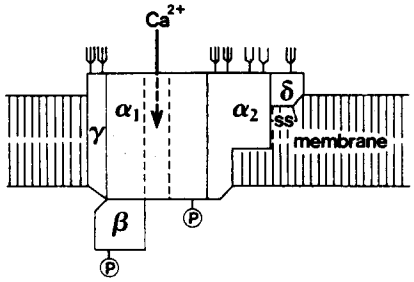


Fig. 1. Schematic representation of the subunit structure of the calcium channel complex. Note that the α_1 subunit contains binding sites for the dihydropyridine-, phenylalklamine- and benzothiazepine-based calcium antagonists [8].

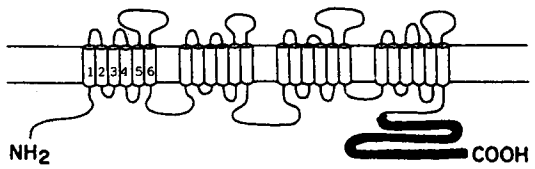


Fig. 2. Schematic representation of the organization of the DHP receptor as it occurs in cardiac and skeletal muscle. The heavily shaded area denotes the carboxy terminus. Note that there are four homologous units, each of which contains six putative transmembrane units.

The characteristics of the calcium antagonist binding sites

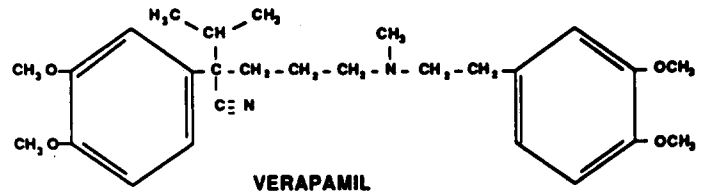
Whilst it is well accepted that the α_1 subunit contains specific high affinity binding sites for the calcium antagonists, the precise location of these sites within the architecture of the subunit is less certain. However, based on the presence or absence of 'use' dependency, it is reasonable to argue that drugs which require the channel to be in the 'open' state and which therefore exhibit 'use' dependency must approach their binding sites by way of an open channel lumen. By contrast, drugs which lack 'use'-dependency or display it to only a small degree probably approach their receptors mainly by way of the lipid-containing membranes, and therefore are usually more lipophilic. The dihydropyridines generally belong to the latter group—with the exception of amlodipine which, presumably because of its charge [19], is slow to penetrate the membrane and hence only slowly reaches equilibrium binding [20]. This may provide this particular calcium antagonist with an advantage because its slow onset of action allows sufficient time for resetting of the baroreceptors, thereby avoiding some of the reflex-induced changes in heart rate which are associated with other dihydropyridine-based antagonists and which often require ancillary treatment in the form of β-adrenoceptor antagonist therapy.

Binding studies using tissue homogenates from heart, smooth muscle, skeletal muscle and brain have clearly established that the 'classical' calcium antagonists—the dihydropyridines, phenylalkylamines and benzothiazepines—occupy distinct but allosterically coupled binding sites on the channel protein [18, 21]. There may, however, be many more binding sites than have been recognized to date. These other sites need not necessarily be associated with the α_1 subunit. Alternatively, they may be located on hitherto unrecognized sites of the subunit. One of the major reasons for arguing that other binding sites may exist is that new ligands are appearing which are chemically unrelated to the prototypes, verapamil, nifedipine and diltiazem (Fig. 3). Although these new ligands show all the pharmacological and physiological characteristics of prototype calcium channel antagonists, they appear not to bind to recognized binding sites in the α_1 complex. Examples of these novel compounds include the compounds pimozide and HOE 166 (Fig. 4).

Novel calcium antagonists with additional antagonist activity

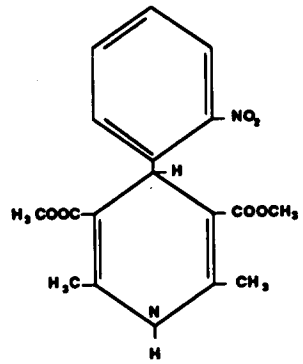
A relatively new development in the field of calcium antagonism, and one which is relevant to the cardiovascular system, is the deliberate search for compounds which can be used to achieve the simultaneous antagonism of other receptors in addition to that of the voltage-sensitive Ca^{2+} channels but within the same dose range as these channels. Two examples serve to illustrate this point: AHR-16303B, a joint calcium channel and 5-HT_2 receptor antagonist [22]; and naftopidil, a joint α_1 adrenoceptor and calcium antagonist, being developed particularly for vascular smooth muscle [23]. The search for new calcium antagonists with ancillary properties may be indicative of the trends in this field.

Before discussing the specific topic of this paper—the involvement of calcium channels in cardiovascular disease as indicated by calcium antagonist therapy—it may be useful to summarize the main trends which are being followed in the search for new compounds. One such trend has already been mentioned, i.e. the search for potent calcium antagonists with ancillary antagonist properties, for example, the combined 5-HT_2 receptor and calcium channel antagonist, AHR-16303B, mentioned above [22]. Other trends include the search for tissue-selective drugs and the development of more potent, long-acting compounds. Nimodipine, a dihydropyride developed for use in the management of patients with stroke or subarachnoid haemorrhage provides an example of the former class [24]. Its use under these conditions depends largely on its selectivity for the cerebral vasculature and its ability to cross the blood–brain barrier. Its relative selectivity for the cerebral vasculature ensures that its introduction is not accompanied by a sustained reduction in peripheral vascular resistance—an effect which would exacerbate damage caused by inadequate cerebral perfusion. Its efficacy depends in part upon its cerebral vasodilator activity, which may improve perfusion in the affected area, and its cytoprotective effect [25]. An example of a long-acting drug is provided by the more recently developed



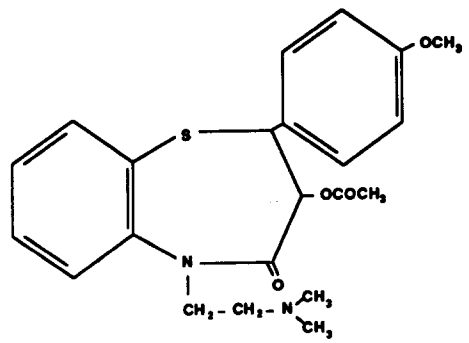
VERAPAMIL

mol wt 454.59



NIFEDIPINE

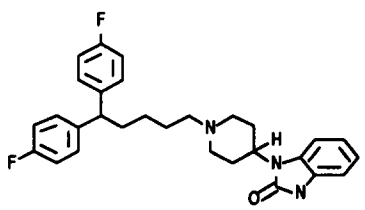
mol wt 346.34



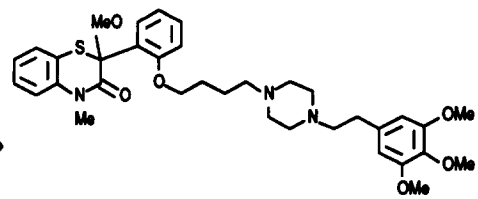
DILTIAZEM

mol wt 414.52

Fig. 3. Chemical structure of the three prototype calcium antagonists verapamil, diltiazem and nifedipine.



Pimozide



Hoe 166

Fig. 4. Chemical structure of two novel calcium antagonists structurally unrelated to the prototype drugs.

dihydropyridine, amlodipine. Amlodipine dissociates only slowly from its receptor [20], a property which facilitates the use of this particular calcium antagonist on a once-a-day basis.

Calcium channels and their involvement in cardiovascular disease

Calcium ion influx by way of the voltage-activated, calcium antagonist-sensitive channels plays a major role in a wide spectrum of cardiovascular disorders ranging from hypertension and certain other peripheral vascular disorders to myocardial infarction, angina pectoris, congestive heart failure and, although possibly indirectly, atherosclerosis. The involvement of L-type Ca^{2+} channel activity in some of these conditions is outlined below.

Hypertension. The primary feature of hypertension is a raised systemic vascular resistance [26]. Any change which may occur in cardiac output must, therefore, be relegated to being a consequence of this resistance and not a cause of the hypertensive state.

Calcium ions play a key role in smooth muscle cell contraction, just as they do in that of cardiac and skeletal muscle. As far as vascular smooth muscle is concerned, "activator Ca^{2+}" is derived from two sources; one extracellular and the other intracellular. The extracellular Ca^{2+} can gain access to the cytosol in several different ways, including by way of the L-type Ca^{2+} channels and, but to a lesser extent, the $Na^+:Ca^{2+}$ exchanger. As far as the intracellularly derived Ca^{2+} is concerned, release is from the sarcoplasmic reticulum and is in response to either the Ca^{2+} which is entering by way of the L-type channels or enhanced inositol triphosphate metabolism, such as that caused by endothelin-1 or α_1 adrenoceptor stimulation. Some vascular smooth muscle cells are more richly endowed with sarcoplasmic reticulum than others. For example, the large conduit vessels contain more reticulum than the small resistance vessels [27]. As a result, the small resistance vessels are more heavily dependent on the influx of Ca^{2+} than their larger conduit counterparts. It is these small resistance vessels which are the primary target of calcium antagonists designed for use as blood pressure-lowering agents.

Of the presently available calcium antagonists the dihydropyridine-based drugs exhibit greater selectivity for the vasculature than their phenylalkylamine- or benzothiazepine-based counterparts. Felodipine and nitrendipine, for example, are relatively selective for the vasculature, whereas the phenylalkylamine-based calcium antagonist verapamil lacks this selectivity and is equipotent in producing Ca^{2+} channel blockade in the heart and vasculature. This relative selectivity of the dihydropyridines is multifactorial in origin. Factors which are involved include: (a) the chemistry of the compounds; (b) the relatively low transmembrane potential difference (−50 mV for smooth muscle, and −85–90 mV for most cardiac muscle cells [28]); and (c) the unusual amino acid content of the α_1 subunit of the Ca^{2+} channel complex, as already described.

The pathophysiology of hypertension. The major causes of hypertension can be divided into at least five categories: (a) the presence of humoral constrictors, some of which (for example, endothelin-1) may directly or indirectly activate L-type Ca^{2+} channels; (b) pressure-induced structural changes in the resistance vessel, causing a reduction in lumen diameter; (c) sympathetic overdrive; (d) autocoids, paracoids and mitogens associated with blood vessels; (e) *a relative increase in the number of functioning L-type Ca^{2+} channels* [29]; and (f) *the prolonged activation of these channels* [30]. Any hypothesis which is aimed at accounting for the hypertensive state must also take into account the following: (a) endothelium-dependent relaxation is impaired [31]; (b) the smooth muscle cells of the vasculature develop a hypersensitivity towards agonists which evoke a contractile response [32]; and (c) cytosolic Ca^{2+} is raised [32]. Under these circumstances, partial inhibition of the L-type Ca^{2+} channels should be expected to cause vasodilation, particularly in hypertensives. As an added bonus, some of the calcium antagonists also slow Na^+ reabsorption in the kidney thereby attenuating any gain in Ca^{2+} which might subsequently occur by way of the $Na^+:Ca^{2+}$ exchanger. By far the most attractive hypothesis which is available at the moment, however, and which is supported by experimental data is that the blood pressure-lowering activity of the calcium antagonists in hypertensives is due to their ability to attenuate the prolonged activation of the smooth muscle L-type Ca^{2+} channel, which appears to be peculiar to the vascular smooth muscle cell of hypertensives [30].

Hence, as far as hypertension is concerned L-type Ca^{2+} channel density is increased [29] and the channels have a prolonged duration of opening [30]. The end result is a raised cytosolic Ca^{2+} and hence an increase in peripheral vascular resistance which will be exacerbated by circulating or locally released agents (angiotensin, endothelin-1, vasopressin) which either activate the L-type Ca^{2+} channels or exacerbate internal Ca^{2+} release.

Myocardial infarction. Ischaemia-induced myocardial injury which progresses to an irreversible loss of structure and function (infarction) is the least desirable consequence of inadequate coronary perfusion. The other two conditions, "stunning" [33] and "hibernation" [34], are reversible conditions, and in contrast to infarction do not result in irreversible calcium overload and cell necrosis [35].

Ca^{2+} influx through the L-type Ca^{2+} channels contributes to the onset of ischaemic conditions in several ways. Firstly, these channels provide one of the routes for Ca^{2+} entry prior to loss of membrane integrity, and at a time when the energy stores are depleted to such an extent [36] as to limit extrusion of the Ca^{2+} across the sarcolemma or its retrieval by the sarcoplasmic reticulum. Under these conditions, cytosolic Ca^{2+} rises (Fig. 5) with the resultant activation of Ca^{2+}-dependent protein kinases, phospholipases and proteases [37]. Secondly, it is the Ca^{2+} which enters by way of the L-type Ca^{2+} channels which dictates largely how rapidly the high energy phosphate reserves become depleted, since it is the Ca^{2+}-induced activation of energy which is the main determinant of myosin ATPase usage under

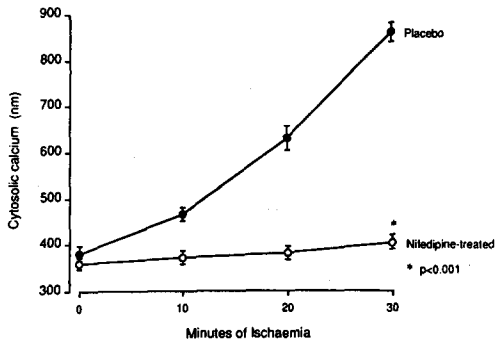


Fig. 5. Cytosolic Ca^{2+} (measured by NMR spectroscopy using F-BAPTA as a Ca^{2+} indicator) in isolated rat hearts made ischaemic for 30 min. Note that in the nifedipine series the drug was added to the perfusion buffer 15 min before the hearts were made ischaemic [46].

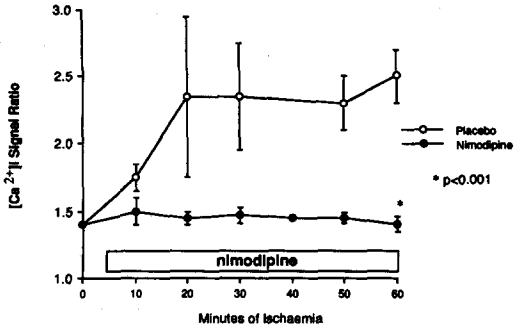


Fig. 6. Effect of nimodipine given as an infusion starting 10 min before occluding the middle cerebral artery in cats (data from Ref. 44; diagram from Ref. 47). Nimodipine was infused at a rate of 1 μg/kg/min. Indo 1-AM was used as the Ca^{2+}-indicator.

these conditions and early during the ischaemic event, when active tension development is still taking place, this is the major source of energy usage.

If Ca^{2+} entry by way of the L-type Ca^{2+} channels plays an important role in the sequence of events which results in infarction and if ionic homeostasis with respect to Ca^{2+} is lost early during the ischaemic episode (Fig. 5), then it is logical to assume that calcium antagonist therapy will be useful only if the drugs are used prophylactically. Two large scale clinical trials [38, 39] have confirmed this assumption.

At this stage, and before considering the criteria which are applicable to the choice of calcium antagonists most suitable for use in the management of patients with ischaemic heart disease, it may be useful to digress and consider the possible involvement of Ca^{2+} channels which are not of the L-type.

Recent evidence [40] indicates that ischaemic episodes may have a profound effect on the Ca^{2+}-storing capacity of the sarcoplasmic reticulum. Not only is this organelle "attacked" by the oxy- and hydroxy-radicals which are generated during prolonged episodes of ischaemia, and subsequent reperfusion [41], the functioning of its Ca^{2+} release channels is also affected [42]. Probably because of ATP depletion, these 'release' channels exhibit a prolonged opening time thereby facilitating efflux into the cytosol of Ca^{2+} which this organelle had already retrieved from the cytosol. Hence, at a time when the sarcolemmal L-type Ca^{2+} channels are still functioning, although their density may be reduced [43], intracellular homeostasis with respect to Ca^{2+} is lost, partly because of insufficient ATP to fuel the Ca^{2+} ATPases responsible for expelling Ca^{2+} from the cytosol and, secondly, because of the failure of the Ca^{2+} release channels of the reticulum to remain closed. Not surprisingly, cytosolic Ca^{2+} rises (Fig. 5) but only in part because of Ca^{2+} entry by way of the L-type Ca^{2+} channels.

Before considering the involvement of the L-type Ca^{2+} channels in other cardiovascular disorders it may be useful to consider briefly the properties a calcium antagonist should possess if it is to be developed for use in the management of patients with ischaemic heart disease. Probably, the drug should: (a) lack marked negative inotropy. Otherwise the pumping capacity of the affected myocardium may be further reduced, with the danger of precipitating cardiac failure. (b) It should show some preference for the coronary vasculature. (c) It should reduce peripheral vascular resistance (to reduce the workload on the heart) but not cause such profound vasodilation as to either trigger a reflex tachycardia or further reduce blood flow through coronary vessels which still remain patent. (d) It should have a long half-life. This is achieved by the use of slow release formulations or, as in the case of amlodipine [20], is due to a slow rate of dissociation from its receptor.

Cerebral ischaemia

Cerebral ischaemia can occur for a variety of reasons including a precipitous fall in blood pressure, emboli arising from thrombi or an ulcerated or ruptured plaque, sustained vasospasm, and cerebral haemorrhage. The term "ischaemic stroke" is often used to describe the acute event in which damage to the brain is immediate. In this respect, the condition is quite different from that caused by a subarachnoid haemorrhage where the damage to the brain is delayed and most probably due to secondary vasospasm [44]. Irrespective of which of these conditions prevails, oxyradicals accumulate and cytosolic Ca^{2+} rises [45] with Ca^{2+} overload, cell death and tissue necrosis the inevitable outcome. Calcium ion flux through Ca^{2+}-selective channels must contribute to this scenario because nimodipine [45], the Ca^{2+} antagonist which shows some selectivity for the cerebral vasculature and which penetrates across the blood–brain barrier, attenuates the rise in cell Ca^{2+} caused by an occlusive event (Fig. 6).

Calcium channels and the atherogenic process

Atherosclerosis is a multifactorial disease which involves not just lipid accumulation but also the localized deposition of collagen, elastin and calcium [48, 49]. The proposed use of calcium antagonists as

anti-atherogenic agents was based initially on the assumption that the Ca^{2+} which accumulates within the mature plaque involves Ca^{2+} entry by way of the L-type channels in the vascular smooth muscle cells [50]. Such an assumption may be correct, but Ca^{2+}-sensitive processes are involved in many of the events of the early stages of lesion formation including monocyte adhesion, release of growth factors, smooth muscle cell proliferation and platelet aggregation. Inhibitors of L-type Ca^{2+} channels are now known to have a direct effect on many of the events which are involved in the formation of atherogenic lesions. For example, they inhibit the migration [51] and proliferation [52] of vascular smooth muscle cells; collagen and matrix synthesis [53]; and cholesteryl ester deposition [54]; and protect against lipid peroxidation-induced injury [55], an event which may play a pivotal role in the uptake of low density lipoprotein during the initial stages of lesion formation [55, 56]. Hence, although inhibitors of L-type Ca^{2+} channels are anti-atherogenic [49] it does not necessarily follow that L-type Ca^{2+} channels are involved in any major way in the early stages of lesion formation, because the presently available inhibitors of L-type Ca^{2+} channels have ancillary properties, some of which may be of therapeutic importance.

The coronary circulation

L-type Ca^{2+} channels are present in the coronary vasculature in both the large conduit vessels and the small resistance arteries [50]. Activation of these channels, irrespective of whether it be direct or the indirect consequence of locally released endothelin [57], probably does contribute to or is the major cause of coronary vasospasm.

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