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COMMENTARY

HOMOLOGOUS AND HETEROLOGOUS REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS

JACQUELINE FERRANTE and DAVID J. TRIGGLE*
School of Pharmacy, State University of New York, Buffalo, NY 14260, U.S.A.

Voltage-dependent Ca^{2+} channels (VDCCs) are now recognized to be a group of membrane proteins, likely members of a large "channel family," distinguished by both electrophysiologic and pharmacologic criteria [1]. The clinically important Ca^{2+} channel antagonists, verapamil, nifedipine and diltiazem, serve to characterize the L class of voltage-dependent Ca^{2+} channel and it is the interaction of these drugs with this class of channel that underlies their therapeutic profile as dominantly cardiovascular agents [2, 3].

VDCCs may be regarded in a number of respects as pharmacologic receptors. Thus, the L class of channel has a discrete set of binding sites with which the primary ligand classes interact. These sites exhibit specific structure–activity relationships and are linked allosterically one to the other and to the functional machinery of the Ca^{2+} channel [3, 4]. At least for the 1,4-dihydropyridine class of drugs both potent antagonists and activators exist [4]. The channels enjoy a selective distribution within the body. Thus, there are significant variations in numbers between different smooth muscles and elements of the cardiovascular system, and there is similarly a heterogeneous distribution within brain areas. VDCCs are modulated by a number of biochemical processes, notably phosphorylation, driven physiologically through hormone and neurotransmitter receptors [3, 5]. Additionally, VDCCs are associated with guanine nucleotide binding (G) proteins that may play a direct role in the channel activation process [6].

In all of these respects VDCCs show a clear analogy to receptors for hormones and neurotransmitters. The regulation of the latter receptors by their endogenous ligands and synthetic analogs, by chronic drug action, during cell development and growth, and in disease states has been well recognized as a characteristic feature of receptor processes [7, 8]. Receptor regulation can occur through a variety of mechanisms; it may occur following short- or long-term exposure to a drug. Changes in receptor metabolism usually occur subsequent to prolonged receptor occupancy, and different mechanisms usually operate during short-term regulation. These latter mechanisms include changes in receptor distribution and phosphorylation state mediated by either endogenous or exogenous protein kinases [9, 10]. In general terms, chronic administration of an agonist or antagonist results in homologous down- and up-regulation, respectively, of receptor number. Receptors may also be regulated heterologously by the actions of drugs or hormones acting at their own distinct receptors. Such regulation may occur through a biochemical modification directed against the regulated receptor, by the alteration of a common element or elements in a transduction pathway shared by two or more receptor systems, or through the genetic machinery via the synthesis of new receptors. A number of disease states are now recognized to be receptor-based and to result from alterations in receptor expression or function at the genetic level including some forms of hypercholesterolemia, at the immune level including myasthenia gravis and Grave's disease, at the hormone level with sympathetic hyper-responsiveness in hyperthyroidism, and at the drug level during withdrawal from cardiovascular agents including propranolol and clonidine.

It is probably that ion channels are regulated similarly. both ion channels and receptors are membrane proteins and both are likely biosynthesized, processed and incorporated into the membrane in a similar fashion. Despite such expectations, regulation may be expressed differently by ion channels and by hormone or neurotransmitter receptors since the latter have well-defined physiologic substrates, hormones and neurotransmitters, whereas endogenous ligands for the 1,4-dihydropyridine class of VDCC (or other category of ion channel) remain to be identified unambiguously [11, 12].

The existing data reveal that, as anticipated, VDCCs are regulated under a variety of conditions including experimental and clinical disease conditions [13–37]. A partial listing of these observations is provided in Table 1. A more detailed compilation and review are forthcoming.†

Up- and down-regulation of 1,4-dihydropyridine binding sites, whole cell channel current, and Ca^{2+} uptake in PC12 cells occur following chronic treatment with nifedipine and S Bay K8644 respectively [15]. This is consistent with observations made in a number of pharmacologic receptor systems and in accord with observations in PC12 cells and chick retinal neurons of down-regulation of 1,4-dihydropyridine binding site number and function following chronic depolarization by elevated K^+ [12, 16]. In

* Address correspondence to: Dr David J. Triggle, School of Pharmacy, 126 Cooke, SUNY, Buffalo, NY 14260.

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Table 1. Regulation of Ca^{2+} channels*

Treatment/Condition	Species	Radioligand/ Tissue	Effects			Ref.
			K_D	B_{max}	Other	
A. Homologous regulation:						
Chronic drug action:						
Oral nitrendipine, verapamil, or diltiazem (28 days)	Mouse	Nitrendipine/Brain	nc	↓ 40% (not diltiazem)	$^{45}Ca^{2+}$ Uptake decreased	13
Nifedipine, i.v. (20 days)	Rat	Nitrendipine/Heart	nc	↓ 49%	↓ 62% β-receptors	14
		Brain	nc	↓ 23%	↓ 65% β-receptors	
Nifedipine		PN 200 110/	nc	↑ 29% Nifedipine		
S Bay K8644		PC12 cells		↓ 24% S Bay K8644		15
Chronic activation:						
K^+ depolarization (4 days)		Nitrendipine/ PC12 cells		↓ 50%	↓ Ca^{2+} influx	16
B. Heterologous regulation:						
Isoproterenol (10 days)	Rat	Nitrendipine/Heart	nc	nc		14
Atropine (23 days)	Rat	Nimodipine/Brain	nc	nc		
DFP (14 days)	Rat	Nimodipine/Brain	nc	nc		
Phenylephrine (6 days)	Rat	Nitrendipine/Heart	nc	↓ 32%	↓ 39% α-receptors	15
Morphine	Mouse	Nitrendipine/Brain	nc	↑ 60%		18
C. Other chronic regulation:						
Ethanol	Rat	Nimodipine/Brain	nc	↑ 50%		19
Lead	Rat	Nitrendipine/Brain	nc	↑ 48%		20
NaCl (21 days)	Rat (SHR/SP)	Nitrendipine/Heart	nc	↑ 65%	↑ Blood pressure	21
D. Hormone regulation:						
Insulin (21 days)	Human	PN 200 110/Muscle	nc	↑ 250%		22
Thyroid:	Rat	Nitrendipine/Heart				
Hyperthyroid (5 days)			nc	↓ 42%	↑ 36% β-receptors	23
Hypothyroid (PTU)			nc	↑ 26%	↓ 23 β-receptors	
Estrogen (4 days)	Rat	Nitrendipine/Uterus	nc	↑ 96%	↑ $^{45}Ca^{2+}$	24
E. Disease states:						
Experimental:						
Hypertension	Rat, SHR (24 weeks)	Nitrendipine/Heart	↑ 55%	↑ 43%		25
Hypertension	Rat, SHR (4–15 weeks)	Nitrendipine/Brain	nc	↑ 21–40% (Striatum, thalamus, hippocampus)		26
Ischemia, 60-min hypoxia	Rat	Nitrendipine/Heart	nc	↓ 14%		27
Ischemia, bilateral ligation	Gerbil	Nitrendipine/Brain (frontal cortex)	↓ 48%	↓ 26%		28
Muscular dysgensis	Mouse	Nitrendipine/ Skeletal muscle	Absent			29
Clinical:						
Muscular dystrophy	Human	Nitrendipine/ Skeletal muscle	nc	nc		29, 30
Cardiomyopathy	Human	PN 200 110/Heart	nc	↑ 25%	nc β-receptors nc Na^+ channel	31

Abbreviations: DFP, di-isopropylphosphorofluoridate; and nc, no changes.

principle, membrane potential, intracellular Ca^{2+}, or both may serve as the regulatory signal(s). Ca^{2+}-mediated channel inactivation occurs with L type VDCCs and may represent a short-term equivalent of regulation. However, intracellular Ca^{2+} does not stay persistently elevated in chronically depolarized PC12 cells [16], suggesting that membrane potential alone may be a sufficient signal for the regulatory event. *In vivo* administration of nifedipine either intravenously or orally to rats results, however, in down-regulation of binding site densities in both heart and brain [13, 14] while *in vivo* administration of nitrendipine in combination with high salt elevates the number of cardiac binding sites in cardiac membranes from stroke-prone spontaneously hypertensive rats [21]. There are obvious differences between the *in vivo* and *in vitro* prococols, notably the absence of any cardiovascular reflex pathways in the latter. However, chronic isoproterenol treatment does not produce any change in cardiac binding site levels, suggesting that *in vivo* factors constitute more than sympathetic activation [14].

Homologous regulation is of potential clinical significance to the issue of withdrawal symptoms following cessation of Ca^{2+} antagonist therapy [2, 32]. However, there are few objective reports of withdrawal phenomena or of the development of tolerance following long-term administration. More extensive studies, both *in vivo* and *in vitro*, are needed to establish any differential sensitivity of cell types and their channels to long-term administration of the Ca^{2+} channel antagonists.

Ca^{2+} channel numbers and functions are clearly regulated by a variety of heterologous influences including insulin and thyroid hormone. The latter is of interest since an opposing regulation of beta-adrenoceptors and Ca^{2+} channels is seen according to thyroid state [23]. However, isoproterenol alone does not regulate Ca^{2+} channels although the anticipated alteration of beta-receptors has been observed [14]. Increases in binding site numbers are observed in morphine-tolerant animals [18] and in animals chronically treated with ethanol [19]. The ethanol effect can be reproduced *in vivo* in PC12 cells [33]. These observations are of interest since the Ca^{2+} channel antagonists are reported to reduce opiate and alcohol withdrawal symptoms consistent with a role, although not necessarily an exclusive one, for neuronal L channels in this hyperexcitability syndrome.

In common with other receptor systems, Ca^{2+} channels are now known to be altered in disease states, both experimental and clinical (Table 1), including hypertension [25, 26], ischemia [27, 28], malignant hyperthermia [34], Lambert–Eaton syndrome [35], muscular dysgenesis [29, 36, 37] and cardiomyopathy [31]. The relationships of these changes to the hypertensive and ischemic states are not well defined. However, the approximately 50% reduction in Ca^{2+} channel binding sites in transverse tubule membranes from skeletal muscle of pigs susceptible to malignant hyperthermia may reflect an adaptation to the abnormal Ca^{2+} release process of this disease. In Lambert–Eaton myasthenic syndrome, a circulating IgG antibody interacts with Ca^{2+} channels at presynaptic motor nerve terminals to produce a generalized clinical picture of myasthenic muscle weakness and disordered autonomic function. However, these antibodies do not cross-interact with cardiac L-type channels. Atrial tissue from human cardiomyopathic patients reveals an approximately one-third increase in binding site density [31]. This increase in Ca^{2+} channels was not accompanied by changes in Na^{+} channels or beta-adrenoceptors, thus arguing against general membrane changes.

Muscular dysgenesis of mice represents a particularly dramatic example of a disease associated with changes in Ca^{2+} channel number. Muscular dysgenesis is a lethal disorder arising from the developmental absence of the Ca^{2+} channel and associated drug-binding sites [29, 36, 37]. It is the absence of the alpha-1 subunit that is detrimental since the alpha-2 subunit is present as are other proteins involved in the storage and release of Ca^{2+}. This defect has been alleviated, in a particularly dramatic fashion, by the microinjection of a plasmid containing complementary DNA encoding the alpha-1 subunit (1,4-dihydropyridine) into cultured dysgenic muscle cells which restores both calcium current and excitation–contraction coupling.

Summary and conclusions. Voltage-dependent Ca^{2+} channels are clearly regulated species under physiologic, pharmacologic and pathologic conditions. This is of importance to questions of chronic drug administration, to hormonal regulation, and to the co-regulation of the channels and their associated modulating receptors. This review has focussed solely on the L class of channel, but it is highly probably that the other classes of Ca^{2+} channel are similarly regulated and thut such regulation contributes to a variety of pathologic disorders.

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