

ASSOCIATE EDITOR: STEPHEN ALEXANDER

# L-Type Calcium Channel Blockers: A Potential Novel Therapeutic Approach to Drug Dependence

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**Abstract**—This review describes interactions between compounds, primarily dihydropyridines, that block L-type calcium channels and drugs that cause dependence, and the potential importance of these interactions. The main dependence-inducing drugs covered are alcohol, psychostimulants, opioids, and nicotine. In preclinical studies, L-type calcium channel blockers prevent or reduce important components of dependence on these drugs, particularly their reinforcing actions and the withdrawal syndromes. The channel blockers also reduce the development of tolerance and/or sensitization, and they have no intrinsic dependence liability. In some instances, their effects include reversal of brain changes established during drug dependence. Prolonged treatment with alcohol, opioids, psychostimulant drugs, or nicotine causes upregulation of dihydropyridine binding sites. Few clinical studies have been carried out so far, and reports are conflicting, although there is some evidence of effectiveness of L-channel blockers in opioid withdrawal. However, the doses of L-type channel blockers used clinically so far have necessarily been limited by potential cardiovascular problems and may not have provided sufficient central levels of the drugs to affect neuronal dihydropyridine binding sites. New

L-type calcium channel blocking compounds are being developed with more selective actions on subtypes of L-channel. The preclinical evidence suggests that L-type calcium channels may play a crucial role in the development of dependence to different types of drugs. Mechanisms for this are proposed, including changes in the activity of mesolimbic dopamine neurons, genomic effects, and alterations in synaptic plasticity. Newly developed, more selective L-type calcium channel blockers could be of considerable value in the treatment of drug dependence.

**Significance Statement**—Dependence on drugs is a very serious health problem with little effective treatment. Preclinical evidence shows drugs that block particular calcium channels, the L-type, reduce dependence-related effects of alcohol, opioids, psychostimulants, and nicotine. Clinical studies have been restricted by potential cardiovascular side effects, but new, more selective L-channel blockers are becoming available. L-channel blockers have no intrinsic dependence liability, and laboratory evidence suggests they reverse previously developed effects of dependence-inducing drugs. They could provide a novel approach to addiction treatment.

## I. Introduction

Calcium is an almost universal second messenger. Over 50 years ago, it was discovered that certain drugs, particularly dihydropyridines, blocked calcium entry into cardiovascular cells (Fleckenstein et al., 1979). The selectivity of these dihydropyridine compounds led to in-depth molecular investigations of voltage-sensitive calcium channels in the cardiovascular system, and for many years, these drugs have

been widely used clinically to treat cardiovascular disorders. At the doses that are used for such treatment, dihydropyridines do not appear to cause overt changes in the central nervous system in humans. The possibility of central effects was therefore originally given little prominence, although some researchers recognized the potential importance (Hoffmeister et al., 1982; Spedding and Middlemiss, 1985). Work in the 1970s had, however, shown that some dihydropyridines, particularly nimodipine, had major effects on CNS

**ABBREVIATIONS:** CNS, central nervous system; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; VTA, ventral tegmental area.

blood flow and were useful in cerebral vasospasm (Towart et al., 1982). This led to a closer examination of the central effects of calcium channel blockers and then to interest in their potential use in the therapy of drug dependence.

The dihydropyridines bind stereospecifically to high-affinity binding sites on particular high-voltage-activated calcium channels, known as L-channels (Bellemann et al., 1983). Most dihydropyridine compounds easily enter the brain and are now known to affect the subtle regulation of neuronal transmission. Recent studies have suggested that dihydropyridine L-channel blockers could have therapeutic value in Parkinson disease (Liss and Striessnig, 2019; Singh et al., 2019) and in Alzheimer-type disorders (Anekonda and Quinn, 2011). Another important health area in which these drugs could be of value is drug dependence, and this is the focus of the current review. The evidence for the involvement of this particular type of calcium channel in dependence, and the potential for the development of novel pharmacological treatments, will be evaluated.

Dependence is a chronic, relapsing disorder characterized by compulsive drug seeking, craving, and continued drug intake despite adverse consequences. Functional neuronal changes in the brain persist for long periods after cessation of the intake of dependence-inducing drugs, resulting in dependence characterized by strong desire for the drug when intake is stopped (craving). The physiological changes that develop from repeated drug intake can be described as an adaptive state, and this terminology will be used throughout this review. The word “adaptive” will be used to describe all changes that occur either in drug actions or in the physiology of the organism, when repeated doses of a drug of dependence are given or taken in; the word is not used in its evolutionary sense.

The categories of dependence-inducing drugs that will be discussed here are alcohol, psychostimulant drugs (i.e., amphetamine, methylamphetamine, and cocaine), opioids, nicotine, benzodiazepines, and barbiturates. The initial target sites of these drugs in the brain differ, but there is a common factor in that they all cause dependence as defined here. Other types of drugs that are abused, such as hallucinogens, that do not cause this type of dependence will not be covered.

## II. Neuronal L-Type Calcium Channels

### A. Calcium Channel Subunits and Subtypes

The calcium channel complexes consist of the pore-forming  $\alpha_1$  subunit, with additional  $\alpha_2\delta$ ,  $\beta$ , and  $\gamma$  subunits, full details of which have been described in an extensive review (Dolphin, 2016). Variations in  $\alpha_1$  subunits result in several isoforms ( $\text{Ca}_v1.x$ ) that differ

in their biophysical properties and locations (Table 1). Auxiliary subunits modify channel activity and are known to be involved in dynamic regulation (Liao et al., 2005; Liao and Soong, 2010; Bock et al., 2011; Campiglio and Flucher, 2015; Zamponi et al., 2015). Alternative splicing produces pharmacological and physiological specificities, including a variant of  $\text{Ca}_v1.3$  with preferential expression in brain, different channel dynamics, and selectivity for drugs (Bock et al., 2011; Huang et al., 2013). Further subdivisions led to the current subtype nomenclature, which now provides a rational isoform categorization, as illustrated (Table 1) (Ertel et al., 2000; Alexander et al., 2019).

### B. Dihydropyridine Binding Sites

Specific dihydropyridine binding sites were originally demonstrated on neuronal cell soma and proximal dendrites (Bellemann et al., 1983; Westenbroek et al., 1990), but there are considerable variations in distribution among neurons (Budde et al., 1998). Evidence indicates that the majority of the binding sites in the brain are on neurons rather than blood vessels (Ricci et al., 2002), with the highest concentrations in hippocampus, amygdala, and substantia nigra and the lowest incidence in the cerebellum and brain stem (Cortes et al., 1984). A recent report that L-channels can now be radiolabeled in vivo in rodents (Firouzyar et al., 2015) may open the way for further investigations. Studies have identified the genes encoding of the  $\alpha_1$  subunits of the  $\text{Ca}_v1.1$  (*CACNA1S*),  $\text{Ca}_v1.2$  (*CACNA1C*), and  $\text{Ca}_v1.3$  (*CACNA1D*) channels (see Table 1), and alterations in these have been implicated in psychiatric disorders (Heyes et al., 2015).

Studies on  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  knockout mice showed that the former channels comprise a large proportion of the dihydropyridine binding in the brain, with  $\text{Ca}_v1.3$  channels forming only about 10%, and low levels of  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.4$  (Sinnegger-Brauns et al., 2009). The use of knockout mice has demonstrated the functional importance in brain of  $\text{Ca}_v1.x$  subtypes; for example, mice lacking hippocampal  $\text{Ca}_v1.2$  channels showed reductions in long-term potentiation (LTP) and in spatial memory (Moosmang et al., 2005). Although it must not be forgotten that

TABLE 1

Subtypes of L-type calcium channel

This table shows the changes in nomenclature, the distribution of subtypes of calcium channel, and the encoding genes. All the listed subtypes were originally known just as “L-type calcium channels” or “L-channels” and then were divided into the subdivisions listed under Previous Name. The current subtype names are listed in the first column of the table. The tissue distribution indicated is not exhaustive, as variations in expression may exist in specific locations, and multiple isoforms can be present in the same cell.

Current Name	Previous Name	Main Distribution	Gene
$\text{Ca}_v1.1$	$\alpha_{1S}$	Skeletal muscle	<i>CACNA1S</i>
$\text{Ca}_v1.2$	$\alpha_{1C}$	Heart, smooth muscle, brain	<i>CACNA1C</i>
$\text{Ca}_v1.3$	$\alpha_{1D}$	Brain, ear, heart	<i>CACNA1D</i>
$\text{Ca}_v1.4$	$\alpha_{1F}$	Retina	<i>CACNA1F</i>

compensatory changes can occur during development in knockout studies, that can complicate interpretation, in this work the Ca<sub>v</sub>1.2 protein was absent only in the adults. Neurons in the mesolimbic system, considered to be of great importance in drug dependence, contain both Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channels (Liu et al., 2014). In recent years, drugs have been developed that have some selectivity for Ca<sub>v</sub>1.2 or Ca<sub>v</sub>1.3 channels (see section IV. B. *Potential Future Drug Candidates* below), but more research is needed.

The dihydropyridine drugs used therapeutically for cardiovascular disorders, such as nifedipine, nimodipine, and isradipine, reduce the conductance of L-channels, but other dihydropyridines can increase calcium conductance, such as the agonist or activator compound Bay K8644 (Schramm et al., 1983). Dihydropyridines are not totally specific for L-channels; some, for example, affect receptors that bind acetylcholine (Fossier et al., 1992). The dihydropyridine binding complex also contains binding sites for phenylalkylamines (e.g., verapamil) and benzothiazepines (e.g., diltiazem) that interact allosterically with the dihydropyridine binding site (Catterall and Striessnig, 1992). Verapamil, and also flunarizine, exerts actions at other sites at concentrations similar to those that block L-channels (Defeudis, 1984; Brown et al., 1986; Taylor and Defeudis, 1986; Hargreaves et al., 1996; Campana et al., 2002). Because of these other actions, the basis of any effects of verapamil or flunarizine in drug dependence models is difficult to identify, so studies on these two drugs are not included in this review. The effects of diltiazem will be covered, as this drug is a selective L-channel blocker, although it acts allosterically on the dihydropyridine binding complex, increasing dihydropyridine binding but reducing L-channel conductance (Yamamura et al., 1982; Tang et al., 2019). The phrase “L-channel ligands” will be used in this review to include dihydropyridine L-channel blockers, diltiazem, and L-channel agonists.

### C. Stereospecificity of Dihydropyridines

The stereospecificity of calcium current blockade by asymmetric dihydropyridines, such as nimodipine, isradipine (originally known as PN-200-110), and Bay K8644, has been important in identifying their actions at L-channels (Towart et al., 1982), since their actions in higher concentrations at other sites do not show such selectivity. The (+) isomer of isradipine has higher affinity for the dihydropyridine binding site and greater action on calcium channels than the (–) isomer (Hof et al., 1986). The (–) isomer of Bay K8644 is a calcium channel agonist, whereas the (+) isomer blocks L-type calcium currents; the racemic compound can demonstrate antagonist effects (Franckowiak et al., 1985).

### D. Voltage Dependence of Dihydropyridine Binding

Binding of dihydropyridine L-channel antagonists is greatly dependent on transmembrane voltage (Greenberg et al., 1986; Hofmann et al., 1999). This has important implications for comparison of the concentrations that produce functional changes in cells and those which displace the binding. Differences have been reported between the voltage activation of neuronal Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channels (Xu and Lipscombe, 2001). Effects of L-channel blockers on neuronal calcium channels are also time-dependent; for example, nifedipine was shown not to alter Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channels opened by a single action potential, but did have effects after continued stimulation (Helton et al., 2005).

### E. Selectivity of L-Channel Blocking Drugs

An examination of the binding of several dihydropyridines to Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channels suggested that nifedipine and nitrendipine exhibited some selectivity for the Ca<sub>v</sub>1.2 isoform (Sinnegger-Brauns et al., 2009), and further studies demonstrated that a specific molecular region may be responsible for Ca<sub>v</sub>1.2 versus Ca<sub>v</sub>1.3 selectivity (Wang et al., 2018). One structural difference postulated to be of importance is that nifedipine and nimodipine have NO<sub>2</sub> substituents that other dihydropyridines (e.g., isradipine, amlodipine, and azidopine) do not possess (Sinnegger-Brauns et al., 2009). Nevertheless, none of the compounds examined in these studies showed more than 3- to 4-fold selectivity for either channel isoform. Tenti et al. (2014), however, have described 5-unsubstituted-C6-aryl-1,4-dihydropyridines with selectivity for Ca<sub>v</sub>1.3 channels. Studies on a group of pyrimidinetrione derivatives showed that changes in the cyclopentyl and arylalkyl substituents appeared selectively to increase or decrease the activity at the Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channel subtypes (Kang et al., 2013), although it has been suggested that this Ca<sub>v</sub>1.3/Ca<sub>v</sub>1.2 selectivity is only modest (Huang et al., 2014). The importance of both naturally occurring variants and manipulations of genotype in elucidating differences between binding sites on Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 subtypes has been emphasized (Berger and Bartsch, 2014).

Studies have shown also that alternative post-transcriptional splicing of Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3 channels modifies their sensitivity to dihydropyridines. Two isoforms in particular have been identified with differential tissue distributions: the Ca<sub>v</sub>1.2a (cardiac) channel has a lower sensitivity to dihydropyridines than the Ca<sub>v</sub>1.2b (smooth muscle) isoform (Liao et al., 2005; Liao and Soong, 2010; Huang et al., 2013). The extent of this difference is dependent on the membrane holding potential, the current-carrying ion, surrounding ion concentrations, and the exact type of cell and drug under study (Zuhlke et al., 1998), but

IC<sub>50</sub> values for isradipine, for example, have been reported to be 32 nmol/l for Ca<sub>v</sub>1.2a and 10 nmol/l for Ca<sub>v</sub>1.2b channels at a holding potential of -80 mV (Lacinova, 2005).

Drug selectivity between channel subtypes and isoforms therefore appears to be possible, and further drug development along these lines could provide greater tissue selectivity. The evidence described in this review suggests that L-channel blockers may have the ability to reduce, or reverse, drug dependence, so if more selective drugs with less cardiovascular action could be developed, these would be a valuable future addition to the treatment of this difficult condition.

### III. Doses, Concentrations, and Pharmacokinetic Aspects

#### A. Concentrations of Dihydropyridines

The concentration range in vitro at which the actions of dihydropyridines are specific for calcium channels is in the high nanomolar/low micromolar range for most compounds, and nonspecific effects (i.e., not via L-channels) occur primarily at concentrations higher than these. Dihydropyridines are highly protein-bound in the periphery and most are highly lipid-soluble compounds and easily cross the blood-brain barrier (Spedding and Middlemiss, 1985; Larkin et al., 1992). Higher concentrations of dihydropyridines have been shown to be required to affect neuronal L-channel currents than are thought to be achieved during therapeutic use of these drugs in cardiovascular disorders (Docherty and Brown, 1986). Evidence suggests that this may apply to many central effects and may involve the different voltage activation of central neurons compared with peripheral tissues (Spedding and Middlemiss, 1985). The production of cardiovascular changes by a particular dihydropyridine dose in vivo does not therefore indicate that the dose was adequate to affect calcium channels in central neurons.

After systemic administration to rodents of doses that produced central effects, such as protection against seizures, direct measurements of brain concentrations of the dihydropyridines have shown levels ranging from 1 to 90 μM (Heffez et al., 1985; Janicki et al., 1988; Dolin and Little, 1989; Larkin et al., 1992). However, systemically administered dihydropyridines have been reported in rodents to produce behavioral effects that lasted longer than might have been predicted from the pharmacokinetic profiles (Sills et al., 1994; Watson and Little, 2002). Correlations have been demonstrated between central brain dihydropyridine concentrations associated with behavioral changes and those that are effective on synaptic

transmission in isolated brain slices (Whittington and Little, 1991; Bailey et al., 1998b).

#### B. Use of Solvents

The low water solubility of the dihydropyridines necessitates the use of solvents for many routes of administration in vivo. Some studies on the effects of dihydropyridines, particularly in early electrophysiology, used alcohol to dissolve these drugs. This may have affected the compounds' actions, since they can potentiate the acute effects of alcohol (Dolin and Little, 1986; Czarnicka and Kubikbogucka, 1993). Demonstration of a lack of effect of an alcohol solvent when administered alone is not therefore proof that it will not cause behavioral and other effects when given in combination with a dihydropyridine.

Even apart from potential interactions between alcohol and dihydropyridines, the levels of alcohol that result when it is used as solvent have been too often ignored, as alcohol has behavioral effects at considerably lower doses than is often thought (Little, 2004). It is crucial in behavioral studies to provide the doses or concentrations of alcohol given if it is used as a solvent. Other solvents may also have pharmacological effects that are likely to affect results. The importance of solvent choice with dihydropyridines was particularly illustrated in a study that compared alcohol, DMSO, or polyethylene glycol to dissolve Bay K8644 and found that both the efficacy and selectivity of Bay K8644 differed according to the solvent (Wu et al., 1992). The solvent used to dissolve L-channel ligands needs to be given greater prominence in publications, particularly in electrophysiology, and the implications and possible interactions need to be discussed, with full details provided (preferably not buried in the supplementary data).

#### C. Pharmacokinetic Aspects

Interpretation of in vivo interactions between L-channel ligands and drugs of dependence naturally needs to include possible alterations in metabolism or distribution of the latter drugs. Evidence on whether dihydropyridines affect alcohol concentrations is conflicting. Nitrendipine and nimodipine were shown not to alter blood or brain concentrations of alcohol in rodents (Dolin and Little, 1989; Whittington et al., 1991), but nifedipine lowered alcohol levels in both humans and rodents (Zacny and Yajnik, 1993; Broadbent, 2013), and isradipine reduced breath alcohol levels in humans (Rush and Pazzaglia, 1998). In addition, the clearance of nifedipine was shown in rats to be reduced by doses of alcohol that would have had behavioral effects (Boje and Fung, 1989). Greatly increased brain amphetamine and methylamphetamine concentrations have been reported after nimodipine administration (Elkins et al., 1993), and nimodipine markedly increased the morphine concen-

tration in serum, but not in brain (Shimizu et al., 2004). In many behavioral studies, there is insufficient consideration of potential pharmacokinetic interactions, and the above results need to be kept in mind during evaluation of the interactions described below. More measurements of drug concentrations, particularly brain levels, are needed.

#### IV. L-Type Calcium Channel Ligands and Drug Dependence

The primary focus of the following sections is the effects of L-channel blockers on the consequences of long-term (chronic) intake of dependence-inducing drugs. Changes in dihydropyridine binding caused by dependence-inducing drugs are also described, as these may be involved in the interactions. Effects of L-channel blockers on single-dose effects of the dependence-inducing drugs will be mentioned only when these are relevant to the dependence.

Properties considered to be of fundamental importance in drug dependence are “reinforcement,” i.e., increased stimulus/response association, and “reward.” Reward is normally considered to include a hedonic (i.e., pleasurable) component that is not necessarily involved in reinforcement (White, 1989); the distinction is often described as the difference between “liking” and “wanting.” The incentive value of a reward is a motivating effect acquired by experience of the drug or other pleasant encounter that stimulates behavior. Negative reinforcement, which motivates drug taking to alleviate the unpleasant feelings of drug withdrawal, is also an important aspect of dependence. Methods used in preclinical studies to measure these drug properties include operant self-administration and conditioned place preference; voluntary consumption of alcohol has also been frequently studied.

In operant self-administration, the animals perform a task such as pressing a lever or poking the nose into a hole, which results in administration of the drug. Results may be affected by drug actions on motor control and alertness, and the many different experimental schedules used can complicate comparisons of data. Valuable results have, however, been obtained, particularly in studies on extinction of self-administration and lever pressing during abstinence phases. Conditioned place preference tests measure the tendency of animals to prefer, or avoid, a location in which they previously experienced the effects of a drug. This method has the advantage that the preference can be measured in drug-free animals, avoiding confounds from motor effects, but it is subject to much variation between experimental designs. In drug discrimination studies, rodents respond on different levers according to whether drug effects are similar to those previously experienced. This gives a measure of a drug's subjective effects, but the

relationship to drug dependence is not fully understood. Voluntary drinking of alcohol has an obvious parallel with the human situation, but alcohol consumption by rodents in free choice situations may be influenced by taste, thirst, and the calories in the alcohol.

Somatic withdrawal signs after cessation of chronic administration of dependence-inducing drugs have frequently been demonstrated to be reduced by blockade of L-channels, as described below. It is noted, however, that the importance of somatic withdrawal syndromes in dependence is debatable, as craving and relapse back into drug taking by human addicts continues long after the overt somatic withdrawal symptoms have subsided. Chronic administration of drugs that cause dependence can also result in the development of either tolerance or sensitization. The relevance of these to the dependence is also controversial, since they do not correlate well with measures of reinforcement. However, these changes do illustrate physiological responses (“adaptations”) to the dependence-inducing drug and, as such, may be relevant to the development of dependence.

##### A. L-Type Calcium Channels and Alcohol

Acute interactions between calcium channel antagonists and alcohol in vivo vary according to the effect measured; concurrent administration of a dihydropyridine increased the ataxic effects and general anesthesia caused by alcohol and reduced locomotor stimulation, but the amnesic actions of alcohol were completely prevented (Dolin and Little, 1986; Czarnicka and Kubikbogucka, 1993; Tampier et al., 1997; Brooks et al., 2002; Balino et al., 2010). It is possible that potentiation of the former effects of alcohol could be problematic for the clinical use of L-channel blockers in alcohol dependence, for example, in overdose, but such interactions would need to be studied later if more specific L-channel blockers are introduced into clinical practice, as their interactions with alcohol may not be the same as the older compounds. The differential acute interactions of current L-channel blockers with alcohol, however, suggest that their effects in models of alcohol dependence, described below, cannot be explained simply by increases or decreases in the acute effects of alcohol.

*1. Reinforcing Effects of Alcohol.* a. *Operant self-administration of alcohol.* Reduction of operant responding for alcohol by L-channel blockers has been reported in several studies. Nimodipine was found consistently to reduce acquisition of operant responding for intravenous alcohol by rats, and low doses slightly increased (and higher doses reduced) previously acquired responding (Kuzmin et al., 1999). In a progressive ratio schedule, nimodipine was shown to decrease the number of reinforcers obtained, and the break points in responding, for low concentrations

(5%–15% v/v) but not high (up to 40%) concentrations of alcohol (Smith et al., 1999b).

An important aspect in relation to potential clinical use is how effective the L-channel blockers are in reducing operant self-administration in alcohol-dependent animals. Recently, mice with a conditional time and tissue-specific  $Ca_v1.2$  gene knockout in fore-brain showed a lack of dependence-induced increase in alcohol-seeking operant behavior and a reduction in cue-induced responding for alcohol 4 weeks after cessation of chronic alcohol treatment (Uhrig et al., 2017). Their data suggest that central  $Ca_v1.2$  channels, rather than  $Ca_v1.3$ , are involved in alcohol-seeking behavior at prolonged intervals after withdrawal. This site might therefore be a target for relapse prevention, if selective  $Ca_v1.2$  channel ligands (see section II. E. *Selectivity of L-Channel Blocking Drugs*) can be utilized.

*b. Conditioned place preference and taste aversion to alcohol.* In a range of studies, no evidence of either place preference or place aversion was found when the L-channel ligands were given alone at doses at which other behavioral effects have been demonstrated in rodents (Calcagnetti et al., 1995; Pucilowski et al., 1995; Chartoff et al., 2006; Liu et al., 2014), and Pucilowski et al. (1996) concluded that conditioned taste aversion to alcohol did not play a major role in the effect of nicardipine in reducing alcohol preference in rats. It has been reported that (–)-nimodipine, but not (+)-nimodipine, caused conditioned taste aversion and that racemic nimodipine produced a conditioned place preference in rats (DeBeun et al., 1996a), and Martin-Iverson et al. (1997) suggested nimodipine caused place aversion in rats. Possible reasons for the difference between these results are the doses and timing: 10 mg/kg and an 80-minute pretreatment time was used by Martin-Iverson et al. compared with 15 mg/kg with a 10-minute pretreatment in the study of DeBeun et al. The design of the apparatus and the solvent used were also different. As described above, most studies have not found such intrinsic actions, and the lack of these is crucial to the potential therapeutic use of L-channel blockers in drug dependence.

One study on conditioned place preference to alcohol found that the short-acting dihydropyridine nifedipine did not alter this conditioning in mice; however, only one dose of alcohol was tested, so interpretation of this result is difficult (Biała and Langwinski, 1996). However, importantly, other studies showed recently that, although systemic administration of isradipine failed to affect expression of conditioned place preference to alcohol on the day of injection, the dihydropyridine prevented previously acquired alcohol place preference (Degoulet et al., 2016). This illustrates the

importance of using a range of testing times in such studies.

Little cross discrimination was found between alcohol and nimodipine (DeBeun et al., 1996b), but both isradipine and nifedipine partially substituted for alcohol in rats (Green and Grant, 1999). In the latter study, nifedipine produced a leftward shift in the dose-response relationship for discrimination between alcohol and water, indicating a potentiating effect, whereas the L-channel activator Bay K8644 caused a corresponding rightward shift.

*c. Voluntary consumption of alcohol.* L-channel blockers have been found by many studies, in a range of different laboratories, to reduce voluntary alcohol drinking in rodents. Nifedipine decreased the preference for alcohol in a free choice between water and 6% v/v alcohol, with no effect on total fluid intake or on blood alcohol concentrations (Engel et al., 1988). Diltiazem was not found to have this direct action in monkeys, but the alcohol consumption was decreased 2 days after the end of the 6 days of diltiazem administration (Rezvani et al., 1991). L-channel blockers reduced alcohol consumption by high alcohol-preferring rats, with isradipine being particularly effective (Fadda et al., 1992). Reductions in food intake were seen only on the first day of L-channel blocker administration, suggesting the effects were specific for alcohol. The dihydropyridine Goe-5438 reduced alcohol drinking in alcohol-preferring P rats, and this effect continued for 2 days after the drug administration (Pucilowski et al., 1992).

A detailed examination of the effects of several dihydropyridines on alcohol consumption by alcohol-preferring rats showed that all reduced preference for a 10% alcohol solution over water, with no changes in total fluid intake, but food consumption was significantly decreased by all the compounds; diltiazem was ineffective (DeBeun et al., 1996c). These authors showed that the (–) stereoisomer of nimodipine was more effective than the (+) isomer, reflecting the stereoselectivity at central dihydropyridine binding sites. Recent research examined effects of intra-accumbal administration of nifedipine and found the L-channel blocker reduced responding for alcohol in non-dependent rats, but the reduction in responding caused in alcohol-dependent animals was not significant (Varodayan et al., 2017).

*2. Alcohol Withdrawal.* Early studies showed L-channel blockers protected against the tremors and convulsions seen in mice in the acute phase of withdrawal from chronic alcohol consumption (Little et al., 1986; Bone et al., 1989), and the calcium channel activator Bay K8644 had an opposing action. The reduction in acute somatic withdrawal signs was stereospecific; the (+) isomer of isradipine had effects, whereas the (–) isomer did not, which suggested the



involvement of specific dihydropyridine binding sites (Littleton et al., 1990). Hyperexcitability in hippocampal CA1 neurons in vitro during alcohol withdrawal was also prevented by dihydropyridine L-channel antagonists, and this effect was stereospecific for isradipine (Whittington and Little, 1991), showing a parallel with the in vivo studies. Furthermore, administration of nifedipine just prior to or during alcohol withdrawal in vivo prevented the increase in c-Fos protein immunoreactivity seen in brain during the subsequent 24 hours (Bouchenafa and Littleton, 1998)

Importantly, the anticonvulsant actions of the dihydropyridines during alcohol withdrawal were selective for alcohol withdrawal seizures, as neither nitrendipine, nimodipine, nicardipine, nor isradipine affected convulsions in mice caused by bicuculline or pentylenetetrazol (Watson and Little, 2002). The electrophysiological studies demonstrated a similar selectivity for protecting against alcohol withdrawal hyperexcitability (Whittington and Little, 1991), suggesting a functional role for the increases in calcium channel conductance reported after withdrawal of alcohol (Whittington and Little, 1993; Molleman and Little, 1997).

Some variability has been seen between individual L-channel blockers on alcohol-induced withdrawal hyperexcitability. Nitrendipine and nimodipine had similar actions in acute alcohol withdrawal in mice, whereas isradipine was more potent, and nicardipine was somewhat less effective (Watson and Little, 2002). Diltiazem, in contrast, increased the severity of the behavioral signs of acute alcohol withdrawal in mice, and the convulsive effects of bicuculline and pentylenetetrazol, as well as the corresponding hyperexcitability in isolated hippocampal slices after alcohol withdrawal in vivo (Watson and Little, 1994; Bailey et al., 1998b). By comparison, in some other models described below, diltiazem had effects similar to the dihydropyridines in its interactions with alcohol.

The anxiety-related behavior seen in rats during acute alcohol withdrawal was found not to be affected by nitrendipine, although tremor was reduced (File et al., 1991); it is possible the anxiety-related component of alcohol withdrawal has a different neuronal origin than the convulsive components. Withdrawal from long-term alcohol intake can also result in hyperalgesia; this was reduced by administration of nitrendipine during the alcohol consumption (Gatch, 2002).

The reduction in central dopamine release seen after withdrawal from drugs of dependence has long been considered to contribute to withdrawal dysphoria. As this change outlasts the acute phase of somatic withdrawal, being seen for example in rats at least 48 hours longer than the somatic withdrawal

signs (Diana et al., 1996), it has been suggested to be involved in the maintenance of dependence. Nimodipine increased extracellular dopamine levels in the shell of the nucleus accumbens in awake rats when administered 10 hours after the last alcohol dose of a chronic treatment (Rossetti et al., 1999). In animals that had not received chronic alcohol treatment, nimodipine had no effect on the dopamine release, indicating that changes in the control of dopamine release by calcium channels take place during alcohol dependence. These authors also observed a reduction in somatic alcohol withdrawal signs after nimodipine treatment.

Memory deficits are a common and serious problem in alcoholics and can continue to be a complication long into abstinence. In rats, administration of nimodipine, either for the last 2 weeks of an 8-month chronic alcohol treatment or as a single administration at withdrawal, prevented the memory deficits measured between 1 and 2 months into the abstinence phase (Brooks et al., 2008). In this study, the prolonged increases in brain corticosteroid caused in rodents by chronic alcohol intake (Little et al., 2008) were also found to be prevented by the nimodipine. Neuronal damage after long-term alcohol treatment has been reported to be reduced by L-channel blockers and worsened by corticosteroids (Nagy et al., 2001; Mulholland et al., 2005). If this effect of an L-channel blocker were seen in humans, and preliminary clinical evidence suggests it may be (Krupitsky et al., 2001), this could have important therapeutic benefits.

*3. Effects of L-Type Calcium Channel Ligands on Adaptive Changes to Alcohol.* When given by repeated injection during chronic alcohol treatment, nitrendipine decreased the development of tolerance to alcohol, as well as the somatic withdrawal signs (Wu et al., 1987; Dolin and Little, 1989; Whittington et al., 1991). These effects did not appear to be due to blood pressure changes, because the peripherally acting antihypertensive drug hexamethonium did not alter alcohol tolerance at a dose that caused a corresponding blood pressure reduction. Other investigators have shown that chronic administration of L-channel ligands can decrease tolerance to other actions of alcohol, such as the anticonvulsant action (Kalynchuk et al., 1992). Further studies showed that the effect of nimodipine in decreasing tolerance to the ataxic actions of alcohol in rats was not seen when the tolerance was context-dependent or when it involved practice on the task (Smith and Little, 2000); the effect therefore appears to be exerted via the changes in the cellular actions of alcohol rather than on conditioning mechanisms.

Although administration of *N*-methyl-D-aspartate (NMDA) antagonists over several hours after



cessation of chronic alcohol intake have also been found to prevent somatic alcohol withdrawal signs (Grant et al., 1990, 1992), there is an important difference from the dihydropyridine actions. When sufficient time was allowed for the removal of an NMDA antagonist from the body prior to withdrawal of the alcohol (72 or 96 hours), the acute alcohol withdrawal hyperexcitability was increased by the concurrent administration of an NMDA antagonist rather than decreased, as in parallel studies using nitrendipine (Whittington et al., 1991; Ripley et al., 1992; Ripley and Little, 1995). This again illustrates the importance of studying a range of different times of administration of drugs in such studies.

Repeated withdrawal from long-term alcohol intake is widely known to increase the severity of somatic withdrawal signs, an effect similar to “kindling” of other types of seizures (Lechtenberg and Worner, 1991; Duka et al., 2003). In laboratory animals, prolonged alcohol intake can also result in delayed development of kindled seizures in response to hippocampal electrical stimulation after alcohol withdrawal; administration of nifedipine during the withdrawal phase reduced this change (Veatch and Gonzalez, 2000).

The prolonged sensitization to drugs of dependence seen after intermittent administration schedules has been suggested to be of importance in the development of addiction (Robinson and Berridge, 1993), although this has been controversial. Nifedipine reduced the acquisition and expression, and diltiazem the expression, of sensitization to alcohol by mice, although the nitrendipine also reduced blood alcohol levels (Broadbent, 2013).

**4. Effects of Alcohol on L-Type Calcium Channels.** Long-term administration of alcohol has been consistently reported to increase dihydropyridine-sensitive binding sites in neuronal tissues. This was demonstrated in cultured neurons (Messing et al., 1986) and during prolonged intake of alcohol in vivo (Dolin et al., 1987). Wu et al. (1987) showed a correlation between binding site increase and the development of tolerance to ethanol. Increases in dihydropyridine binding in vivo in mice during the acute withdrawal phase correlated with the behavioral signs of alcohol withdrawal (Watson and Little, 1999a). That such raised binding is functional was shown by increases in dihydropyridine-sensitive calcium currents in hippocampal neurons after withdrawal from chronic alcohol treatment in vivo (Huang and McArdle, 1993; Whittington et al., 1995; Molleman and Little, 1997). It has been shown to be mediated by protein kinase (Gerstin et al., 1998).

More recent studies on calcium channel subtypes have confirmed and extended these results. Increased expression of the  $Ca_v1.2$  and  $Ca_v1.3$ , and  $\alpha2\gamma1$

subunits of L-type calcium channels, were seen in mouse cerebral cortex tissue immediately after cessation of chronic alcohol treatment (Katsura et al., 2005). Increases in mRNA and protein levels of  $Ca_v1.2 \alpha1$  and  $Ca_v1.3 \alpha1$  subunits in the inferior colliculus were reported after withdrawal from chronic alcohol treatment, and detailed examination of the time course showed that the  $Ca_v1.3$  subunit changes paralleled the behavioral signs of alcohol abstinence, peaking at 24 hours after withdrawal (N’Gouemo, 2015). When later phases of alcohol abstinence were studied, an increase in  $Ca_v1.2$  mRNA concentration in the amygdala and hippocampus of alcohol-dependent rats at 21 days of abstinence was demonstrated, with no changes in  $Ca_v1.3$  mRNA. This was associated with increased L-channel current amplitudes in hippocampal pyramidal neurons, demonstrating the functional significance of the mRNA changes (Uhrig et al., 2017).

**5. Clinical Studies on L-Channel Ligands and Alcohol Dependence.** Clinical studies on the effects of L-channel ligands in alcohol dependence so far have been few in number, and the results have not been consistent. A small open study of the effects of nimodipine (120 to 220 mg per day for 3 weeks) in alcoholics demonstrated a significant reduction in the symptoms of alcohol withdrawal and good toleration (Altamura et al., 1990). However, in a small pilot study in detoxifying alcoholics receiving four 60-mg doses of nimodipine, incidences of confusional states and one generalized seizure were reported (Deckert et al., 1992). A placebo-controlled study, also using four 60-mg doses of nimodipine, did not see significant reduction of moderate alcohol withdrawal symptoms in patients who were also being treated with clomethiazole to reduce withdrawal problems (Banger et al., 1992). Published preliminary data indicated a short-lasting effect of nifedipine, 60 mg per day, in decreasing relapse drinking in alcoholics (Samocho-wiec, 2000). Another study, primarily examining the effects of ketamine in alcoholics abstinent for at least 1 month, found that nimodipine (90 mg) improved memory function in these patients (Krupitsky et al., 2001).

### *B. L-Type Calcium Channels and Psychostimulant Drugs*

The acute behavioral actions of the psychostimulant drugs cocaine, amphetamine, and methylamphetamine have been reported to be reduced, increased, or unchanged by administration of L-channel blockers (Grebb, 1986; Pani et al., 1990; Ansah et al., 1993; Rosenzweig-Lipson and Barrett, 1995), so the effects of the latter drugs in the dependence cannot be explained by a consistent acute interaction. In addition, the pharmacokinetic interactions with nimodipine (Elkins et al., 1993), described in section III. C.

*Pharmacokinetic Aspects*, will almost certainly have influenced results from behavioral tests. It is important to determine whether similar pharmacokinetic interactions occur with other L-channel blockers, as it could, for example, mask antagonist effects of L-channel ligands on the properties of amphetamine.

*1. Reinforcing Effects of Psychostimulant Drugs.* *a. Operant self-administration of psychostimulant drugs.* Several studies have shown that L-channel blockers reduce operant self-administration of cocaine, but there have been fewer studies on amphetamine. Nose-poke responding by mice to receive a set amount of intravenous cocaine was decreased by isradipine and by nimodipine (Kuzmin et al., 1992b). In a continuous reinforcement design, isradipine significantly increased the responding pattern of previously trained rats for cocaine administration (Martellotta et al., 1994). Although this might appear to contradict the results of Kuzmin et al., Martellotta et al. concluded that isradipine decreased the reinforcing effect of cocaine because a similar pattern was seen when saline was substituted for previously available cocaine. Importantly, this isradipine effect was stereospecific, paralleling the effects of isradipine isomers on calcium conductance. A similar conclusion was reached by Kuzmin et al., who found that nimodipine reduced rats' responding for low and medium doses of cocaine, whereas the L-channel activator Bay K8644 showed opposite effects (Kuzmin et al., 1996).

Anderson et al. (2008) measured the reinstatement of responding for cocaine after extinction of the response, an effect considered to be of considerable relevance to the "cocaine seeking" seen in dependent humans. Systemic administration of diltiazem before a priming dose of cocaine reduced the resultant reinstatement of cocaine responding. The same pattern was seen after microinjection of diltiazem into the shell of the nucleus accumbens, indicating the location of the interaction, whereas diltiazem had no effect on "food seeking" in a similar paradigm. Further important data were provided by Addy et al. (2018), who showed that in rats after 10 days of abstinence from cocaine, after acquisition of self-administration, either systemic or ventral tegmental area (VTA) administration of isradipine prevented cue-induced cocaine seeking but not sucrose seeking. The former effect was accompanied by increases in both tonic and phasic dopamine release in the nucleus accumbens, suggesting a dopaminergic basis. These data are consistent with the earlier results of Rossetti et al. (1999), who demonstrated increases in dopamine after nimodipine administration only in alcohol-dependent rats. This is of considerable therapeutic importance, since an addiction treatment would be

most valuable if it was effective when given during abstinence.

In contrast to the above results, no effects of nimodipine or diltiazem on cocaine self-administration by squirrel monkeys were found at doses that reduced the pressor effects of cocaine (Schindler et al., 1995). Comparison of the doses of calcium channel blockers used in different species is always complex, and in the latter study, these drugs were given intravenously, whereas the intraperitoneal route has been used in rodents.

*b. Conditioned place preference to psychostimulant drugs.* Several studies have demonstrated reduction by isradipine of conditioned place preference induced by cocaine. Cocaine-induced place conditioning was reduced by administration of isradipine prior to conditioning (Pani et al., 1991), and isradipine given during the conditioning completely blocked the development of amphetamine-conditioned preference (Pucilowski et al., 1995). Diltiazem, however, given by microinjection into the ventral nucleus accumbens shell was found to increase the ability of a subthreshold dose of cocaine to establish place preference, whereas application to dorsal accumbens had no effect (Chartoff et al., 2006).

More recent studies have investigated the location of the role of L-channel isoforms in the different stages of psychostimulant-induced conditioned place preference. Intracerebroventricular administration of nifedipine dose-dependently reduced the development of methamphetamine- and cocaine-induced conditioned place preference (Shibasaki et al., 2010). Bilateral intra-VTA injection of isradipine before conditioning sessions prevented the development of conditioned place preference to cocaine (Degoulet et al., 2016). In this study, systemic administration of isradipine failed to affect expression of place preference to cocaine on the day of injection, but importantly, testing on the subsequent 2 days showed the dihydropyridine prevented previously acquired cocaine place preference. A similar effect was seen when isradipine or a more  $\text{Ca}_v1.3$  selective cyclopentylpyrimidine L-channel antagonist was injected into the VTA. Mice with a single point mutation of the  $\alpha 1$  subunit of  $\text{Ca}_v1.2$  channels, that reduced sensitivity to L-channel ligands without affecting  $\text{Ca}_v1.2$  function and expression, were used to study cocaine-induced place preference. (Martinez-Rivera et al., 2017). Nifedipine infusion into the VTA reduced, and corresponding Bay K8644 infusion increased, the established place preference, indicating a role for  $\text{Ca}_v1.3$  channels in the pre-limbic cortex. However, evidence has been reported for the role of  $\text{Ca}_v1.2$  channels in reinstatement of psychostimulant conditioned place preference. Focal deletion of  $\text{Ca}_v1.2$  channels in this region prevented both cocaine- or stress-induced reinstatement without

affecting acquisition or extinction. In addition, intraperitoneal injection of isradipine prior to the reinstatement tests prevented both types of reinstatement (Bavley et al., 2020). Although different channel subtypes may be involved in different brain areas, an important conclusion from these data, as from the self-administration studies described above, is that established psychostimulant conditioning can be counteracted by subsequent administration of an L-channel blocker. This has clear relevance to the potential use of the latter type of drug to treat dependence (see section *V. C. Do L-Type Calcium Channel Ligands Reverse Established Changes in Drug Dependence?*).

**2. Psychostimulant Drug Withdrawal.** Effects of L-channel ligands on withdrawal behavior seen after cessation of prolonged administration of psychostimulant drugs have been little studied, in contrast to the many studies on psychostimulant sensitization, which are described below. However, in mice during the 2 weeks after cessation of 2 weeks of treatment with amphetamine, nimodipine was found to reduce withdrawal-induced increases in anxiety-related behavior (measured using a modified elevated plus maze) and in depression-related behavior (measured by the forced swim test) (Biała et al., 2014).

**3. Effects of L-Channel Ligands on Adaptive Changes to Psychostimulant Drugs.** Sensitization to psychostimulant drugs seen after repeated administration has aroused considerable interest, and several groups have shown that L-channel blockers reduce the development and expression of sensitization to cocaine and to amphetamine or methamphetamine (Karler et al., 1991; Burger and Martin-Iverson, 1994; Pierce et al., 1998). Repeated administration of Bay K8644 into the VTA caused sensitization to the locomotor stimulant actions of cocaine (Licata et al., 2000), whereas corresponding administration of diltiazem increased the acute locomotor effects of cocaine but reduced sensitization development (Licata et al., 2004).

Both  $Ca_v1.3$  and  $Ca_v1.2$  channels appear to be involved in psychostimulant sensitization. The role of  $Ca_v1.3$  channels in amphetamine sensitization was indicated by the results of Giordano et al. (2006), who found that knockout mice with functional  $Ca_v1.3$  channels, that lacked dihydropyridine sensitivity, did not exhibit sensitization after repeated amphetamine. Further studies showed that the  $Ca_v1.3$  subtype of L-channel is necessary for the development of psychostimulant sensitization, whereas the  $Ca_v1.2$  subtype mediates the expression of sensitization (Giordano et al., 2010). The latter authors demonstrated that the activation of the  $Ca_v1.3$  channels following a single injection of amphetamine was sufficient to mediate sensitization, whereas  $Ca_v1.2$ -dependent blunting of the transcription factor cAMP-responsive element binding protein activation was seen only after a 14-

day drug-free period. They suggested that a molecular switch from  $Ca_v1.3$  channels to  $Ca_v1.2$  channels mediates the initial versus the prolonged changes during psychostimulant administration. Subsequent work from this group, however, showed that  $Ca_v1.3$  calcium channels do mediate a long-term adaptation in D2 dopamine receptor-mediated glutamate transmission in the dorsal striatum after repeated cocaine administration after a protracted drug-free period (Schierberl et al., 2012). In addition, repeated cocaine injections followed by 3- or 21-day abstinence was found to reduce the dopamine D2-receptor-mediated modulation of L-channel activity in nucleus accumbal medium spiny neurons (Perez et al., 2011). Recently, conditional knockout mouse lines lacking *Cacna1c* exhibited reduction in both the acute locomotor stimulant effects of amphetamine or cocaine and sensitization to such effects. Use of a specific dopamine-releasing drug showed these results were mirrored by removal of *Cacna1c* in the VTA but not the nucleus accumbens (Terrillion et al., 2017). Systemic pharmacological blockade with nimodipine paralleled this change, indicating the differences in the knockouts were not due to compensatory mechanisms.

Tolerance to the behavioral effects of psychostimulant drugs is little studied, as most studies have focused on sensitization, although tolerance, and crosstolerance, do occur to some of their effects (Little and Rees, 1974). Crosstolerance between the effects of amphetamine and nicotine on anxiety-related behavior was shown to be decreased by nimodipine or diltiazem when either of these drugs was given prior to each injection of amphetamine or nicotine (Biała and Kruk, 2008).

**4. Effects of Psychostimulant Drugs on L-Type Calcium Channels.** Upregulation of dihydropyridine binding sites has been reported in rat brain at 1 and 3 days after repeated administration of psychostimulant drugs (Shibasaki et al., 2010; Napier et al., 2014). Increased total cell surface expression of  $Ca_v1.2$   $\alpha 1C$  subunits in pyramidal neurons was demonstrated in the medial prefrontal cortex at 3 and 21 days after repeated cocaine administration, plus an increase in total expression of  $Ca_v1.3$   $\alpha 1D$  subunits at 21 days (Ford et al., 2009). These authors did not see such changes in the motor cortex, and they suggested their data indicated involvement of L-channels in the hypersensitivity of the prefrontal cortex to cocaine or to cocaine cues.

An increase in  $Ca_v1.2$  mRNA and protein levels, with no change in  $Ca_v1.3$ , was reported in rat ventral tegmental dopamine neurons 24 hours after repeated amphetamine administration (Rajadhyaksha et al., 2004). However, repeated administration of methylamphetamine or cocaine was found to increase protein levels of both  $Ca_v1.2$  and  $Ca_v1.3$  in the frontal

cortex and limbic forebrain 24 hours after the last psychostimulant dose (Shibasaki et al., 2011). Extinction, but not acquisition, of conditioned place preference for cocaine was found to increase  $Ca_v1.2$  mRNA and protein in hippocampal postsynaptic density fractions (Burgdorf et al., 2017). In addition, rats with established cocaine self-administration behavior had higher total and membrane protein expression of  $Ca_v1.2$  and lower expression of  $Ca_v1.3$  in the dorsolateral striatum than rats that did not exhibit such behavior (Shen et al., 2018). Another investigation showed that 48 hours of exposure of SH-SY5Y dopaminergic cells to methylamphetamine caused specific increases in expression of the *CACNA1C* gene and the density of L-channels (Andres et al., 2015).

Although increases in L-channels and/or mRNA have been consistent after repeated psychostimulant drug administration, there appear to be differences between brain areas and channel subtypes. Functional upregulation of L-channels, however, was demonstrated by increased responsiveness to excitatory stimuli of calcium currents, particularly those mediated by L-channels, in rat medial prefrontal cortex neurons at 3 or 21 days after cessation of chronic cocaine administration (Nasif et al., 2005).

**5. Clinical Studies on L-Type Calcium Channel Ligands and Psychostimulant Drugs.** Although the effects of L-channel ligands have been consistent in preclinical studies on psychostimulants, as described above, clinical data so far have been somewhat contradictory. The subjective effects of cocaine and amphetamine in humans were reported not to be affected by diltiazem (Rowbotham et al., 1987; Fabian and Silverstone, 1997), but those induced by cocaine were reduced by nifedipine (Muntaner et al., 1991). Isradipine reduced some subjective effects of, and craving for, methylamphetamine in nondependent volunteers (Johnson et al., 1999). Further studies, however, showed only minor effects (Johnson et al., 2005). These authors also found that isradipine had no effect on the stimulant action or cognitive effects of cocaine and that sustained-release isradipine did not reduce the preference for cocaine in abstinent cocaine-dependent individuals (Johnson et al., 2004). The authors noted, however, that the isradipine dose was limited by potential cardiovascular side effects (see discussion of clinical dihydropyridine doses in section IV. A. *Summary of L-Channel Ligand Interactions in Drug Dependence*).

### C. L-Type Calcium Channels and Opioids

Early studies showed that L-channel ligands can increase some acute effects of opioids, such as antinociception (Benedek and Szikszay, 1984; Hoffmeister and Tettenborn, 1986; Antkiewicz-Michaluk et al., 1993; Michaluk et al., 1998), and decrease other acute actions, hyperthermia, respiratory depression, and

euphoria (Benedek and Szikszay, 1984; Pillai and Ross, 1986). These data suggest the effects of dihydropyridines in opioid dependence described below were not solely due to increases or decreases in the acute actions of the opioids. An important, and often neglected aspect, however, was highlighted by the results of Hodoğluğil et al. (1996), who showed that L-channel blockers potentiated morphine's antinociceptive actions during the light-phase period, but had little effect during the dark phase, when laboratory rats and mice are normally active.

**1. Reinforcing Effects of Opioids.** *a. Operant self-administration of opioids.* Isradipine and nimodipine decreased intravenous self-administration of morphine by mice (Kuzmin et al., 1992b). Later studies showed a rightward shift of the dose-response relationship for morphine in a nose-poke paradigm with nimodipine, whereas the L-channel activator Bay K8644 caused a leftward shift. The authors concluded that nimodipine reduced morphine reinforcement, since the opioid receptor antagonist naloxone, in the same paradigm, had a similar effect (Kuzmin et al., 1994).

*b. Conditioned place preference to opioids.* Kuzmin et al. (1992a) found that concurrent administration of isradipine almost completely prevented the acquisition of place conditioning to morphine. This effect was dose-dependent and was seen at isradipine doses below those that potentiated the antinociceptive actions of morphine. In agreement with these results, nifedipine prevented the ability of morphine to produce a conditioned place preference (Biała and Langwinski, 1996). That L-channel ligands reduce the reinforcing actions of opioids was also suggested by the reduction by isradipine of morphine-induced hypothalamic self-stimulation (Bespalov and Zvartau, 1995).

**2. Opioid Withdrawal.** Administration of L-channel blockers has been found by many research groups to reduce both antagonist-precipitated and spontaneous signs of opioid withdrawal. Single doses of nimodipine decreased the agitation, weight loss, and diarrhea seen in rats during the acute phase of naloxone-precipitated withdrawal from chronic morphine treatment, whereas the L-type calcium channel activator Bay K8644 had some potentiating effect (Bongianni et al., 1986; Ramkumar and Elfakahany, 1988; Barrios and Baeyens, 1991). That the dysphoric effects of opioid withdrawal may also be reduced by L-channel ligands was suggested by the demonstration that nimodipine administration just prior to giving the opioid antagonist significantly reduced place aversion produced in rats by naloxone-precipitated withdrawal from chronic opioid administration (Budzynska et al., 2012).

L-channel blockers have also been found to prevent the opioid withdrawal syndrome when given chronically. No signs of opioid abstinence were seen when rats were treated chronically with morphine plus nifedipine, whereas rats given morphine alone showed characteristic somatic withdrawal signs when these were measured 24 hours after the last treatment (Antkiewicz-Michaluk et al., 1993). Nifedipine has a short duration of action compared with other dihydropyridines mentioned, so it is unlikely the reduced withdrawal severity was due to a direct acute action of the dihydropyridine. Other studies showed a reduction in morphine withdrawal signs when nimodipine was given concurrently with the opioid until 24 hours prior to testing (Michaluk et al., 1998). Nimodipine was also shown to prevent memory loss in mice during morphine withdrawal, whether given acutely on withdrawal or chronically with the morphine (Vaseghi et al., 2012, 2014). These data have important clinical relevance.

Changes in single unit recordings from locus coeruleus neurons, as well as acute behavioral signs produced by naltrexone in morphine-dependent rats, were also prevented by a single dose of nimodipine prior to testing (Krystal et al., 1996). In this study, nimodipine was dissolved in 50% alcohol. The volume of injection is not stated, but calculations suggest the alcohol dose could be 0.1–1 g/kg alcohol, doses known to have behavioral effects (Little, 2004). Although the vehicle was stated not to have effects alone, and even if smaller amounts of alcohol were used, acute interactions with the nimodipine could still have occurred (see section III. B. *Use of Solvents*).

*3. Effects of L-Type Calcium Channel Ligands on Adaptive Changes to Opioids.* Calcium channel antagonists have long been known to decrease the development of opioid tolerance (Wu et al., 1987; Contreras et al., 1988, 1993; Ruiz et al., 1993). Reversal of established opioid tolerance was demonstrated after intracerebroventricular administration of nifedipine into mice previously implanted with morphine pellets (Smith et al., 1999a). In addition, concurrent administration of diltiazem with morphine reduced opioid tolerance (Verma et al., 2001). In contrast, concurrent administration of a low dose of nimodipine during chronic morphine treatment apparently increased the tolerance to morphine's antinociceptive effects (Zharkovsky et al., 1999). The authors suggested the difference from other results might be due to the fact that measurements were made at a time when the dihydropyridine would have cleared from the brain rather than when it was still present; potential metabolic interactions were excluded.

Supersensitivity to opioids can be seen under certain conditions after withdrawal from chronic opioid administration. The development, but not the

expression, of sensitization to morphine-induced locomotor hyperactivity was reduced by nimodipine and by nifedipine (Zhang et al., 2003). At the doses used in this study, nimodipine reduced the acute effect of morphine, but nifedipine had less effect; this may partially explain the results in the sensitization studies.

*4. Effects of Opioids on L-Type Calcium Channels.* Chronic administration of opioids has consistently been found to increase the density of central dihydropyridine binding sites (Saito et al., 1985; Ramkumar and Elfakahany, 1988; Antkiewicz-Michaluk et al., 1990; Diaz et al., 1995), and anatomic differences have been reported. Scatchard analysis of isradipine binding showed increased maximum specific binding values for binding in the cerebral cortex and the mesolimbic region but not in the cerebellum; tissues were taken 1 hour after the last morphine dose. In the former brain regions, both the  $Ca_v1.2$  and  $Ca_v1.3$  isoforms of L-channels and the  $\alpha2/\delta1$  subunit were upregulated (Shibasaki et al., 2007). However, in mid-brain regions of mice, the L-channel  $Ca_v1.3$  protein, but not the  $Ca_v1.2$  protein or phosphorylation state, was significantly decreased during a chronic morphine treatment that caused tolerance (Haller et al., 2008). A single dose of morphine did not change the expression of any of the channel subunits, suggesting the change was related to opioid dependence.

Studies have also shown that concurrent administration of nimodipine prevented the increases in dihydropyridine binding caused by chronic opioid administration (Michaluk et al., 1998). Coadministration of nifedipine chronically with morphine prevented both the acute somatic withdrawal signs and the corresponding changes in brain nitric oxide synthetase (Vitcheva and Mitcheva, 2004). This pattern is similar to those seen in the increases in dihydropyridine binding after chronic alcohol treatment described above (section IV. A. *4. Effects of Alcohol on L-Type Calcium Channels*).

*5. Clinical Studies on L-Type Calcium Channel Ligands and Opioids.* The above preclinical results suggested that L-channel blockers might be of value clinically in opioid dependence, and some human studies have supported this. In a small open study in patients with cancer pain who had previously needed successive increments of morphine dose, nimodipine (120 g per day) reduced the daily dose of morphine required in 16 out of 23 patients (Santillan et al., 1994). In a later double blind study, the same dose of nimodipine was effective in reducing both the required morphine dosing and the escalation of opioid dose in patients with cancer, thus demonstrating reduction of previously established tolerance to morphine (Santillan et al., 1998). Measurement of the plasma concentrations of morphine and its metabolites showed no effect of nimodipine on these levels, and no effects of the dihydropyridine were seen on

respiratory depression by the opioid or the reported side effects. Other studies have not shown such interactions. Neither nimodipine (60 mg) nor diltiazem (30 mg) altered measures of pain sensitivity after morphine administration in healthy volunteers (Hasegawa and Zacny, 1997). However, the dose of nimodipine used in this study was lower than in the studies of Santillan et al. described above, so it is possible a potentiation of analgesia would have been seen with higher doses, although this could also have led to greater side effects. In addition, as Santillan et al. suggest, the patients in the cancer trial had been using opioids for pain control for some considerable time, and the calcium channel blockers could have been more effective because of this (Santillan et al., 1998).

In an early study of the effects of nifedipine in the treatment of naltrexone-precipitated withdrawal in morphine-dependent patients, symptoms of withdrawal appeared to be decreased, but the trial was halted after two patients developed severe confusion after naltrexone was given (Silverstone et al., 1992). The authors suggested that the problem could have been caused by an unopposed release of noradrenaline. Oliveto et al. (2004) studied the effects of calcium channel antagonists on the behavioral effects of naloxone in methadone-maintained volunteers trained to distinguish between low-dose naloxone and placebo. Isradipine significantly attenuated naloxone-induced responding and reduced the increases in opioid receptor antagonist ratings and ratings of sedation produced by naloxone. The authors concluded that isradipine decreased some of the behavioral consequences of naloxone administration to opioid-dependent humans.

#### D. L-Type Calcium Channels and Nicotine

Considerably fewer studies have been carried out on the involvement of L-channels in the actions of nicotine, and in nicotine dependence, than for the other dependence-inducing drugs described above, but there have been some intriguing results. Acute interactions between nicotine and L-channel blockers, such as the reduction in nicotine-induced antinociception caused by L-channel blockade (Damaj and Martin, 1993), need to be taken into account in their interpretation.

**1. Reinforcing Effects of Nicotine.** *a. Operant self-administration of nicotine.* Isradipine reduced self-administration by mice via nicotine-contingent nose-poke responses, stereospecifically and in a dose-related manner (Martellotta et al., 1995). These authors concluded that isradipine reduced the reinforcing properties of nicotine and that it might be useful in treating nicotine dependence. In line with this conclusion, nicotine discrimination in a two-lever, food-motivated, operant task was prevented by isradipine (Schechter and Meehan, 1992), although the

isradipine dose, 15 mg/kg, was relatively high for this particular dihydropyridine in rodents.

*b. Conditioned place preference to nicotine.* Place conditioning to nicotine in mice has been reported to be prevented by pretreatment with either nimodipine or diltiazem (Biała, 2003). A more detailed study used  $Ca_v1.3$  knockout and  $Ca_v1.2$  knockout mice: the specific mutation in the latter removed only the dihydropyridine binding domain with normal channel function, whereas the former exhibited a range of abnormalities. However, although both knockouts and the wild type displayed nicotine-conditioned place preference, systemic pretreatment with nifedipine prevented the place preference only in the wild-type and the  $Ca_v1.3$  knockout mice, not in the  $Ca_v1.2$  knockout animals (Liu et al., 2017). No conditioned place preference or aversion was seen with nifedipine alone. The authors concluded that the data indicated the  $Ca_v1.2$  channel subtype is necessary for the reinforcing effects of nicotine, whereas the  $Ca_v1.3$  subtype may be involved but is not necessary.

**2. Nicotine Withdrawal.** In mice, nimodipine and diltiazem were found to attenuate mecamylamine-precipitated somatic nicotine withdrawal signs (Biała and Weglinska, 2005). Nimodipine, at low doses (1 and 2 mg/kg), reduced somatic signs of nicotine withdrawal in mice at 18–24 hours after the last nicotine dose, but did not alter the corresponding conditioned place aversion or anxiety-related behavior (Jackson and Damaj, 2009). In addition, higher doses of nimodipine (5 and 10 mg/kg) reduced the place aversion in rats produced by mecamylamine-induced nicotine withdrawal (Budzynska et al., 2012). Similarly, nimodipine, given as single intraperitoneal injections of 10 and 20 mg/kg prior to testing, reduced the anxiety-related behavior, memory impairment, and hyperalgesia seen on the 7th and 14th day after withdrawal from 14 days nicotine treatment (Biała et al., 2014).

**3. Effects of L-Type Calcium Channel Ligands on Adaptive Changes to Nicotine.** Acute tolerance to the hypothermia and motor impairment produced by nicotine was prevented by coadministration of nimodipine (Damaj et al., 1996), and chronic administration of nimodipine prevented tolerance to the antinociceptive action, whereas Bay K8644 had the opposite effect (Damaj, 2005). A similar pattern was seen for the increase in anxiety-related behavior produced by nicotine, tolerance to which was reduced by nimodipine and by diltiazem (Biała and Budzynska, 2006).

The L-channel antagonists were also found to reduce locomotor sensitization to nicotine (Biała, 2003; Biała and Weglinska, 2004). Importantly, when nifedipine was given during a 7-day abstinence phase, after 14 days of nicotine treatment, it prevented the subsequent sensitization to nicotine (Bernardi et al., 2014). These data have considerable implications for therapy.

#### 4. Effects of Nicotine on L-Type Calcium Channels.

Changes in central dihydropyridine binding have been reported after chronic nicotine treatment, but the effects appear to be somewhat more complex and less consistent than those described above for alcohol and opioids. No changes were found in central mouse dihydropyridine binding 12 hours after the last of 10 days of chronic nicotine injections (Damaj, 1997), but increases in RNA expression for an L-channel subunit and calcium influx were seen in primary cultures of mouse cerebral cortical neurons after 72 hours of nicotine exposure (Katsura et al., 2002). Increased expression of  $Ca_v1.2$ ,  $Ca_v1.3$ , and  $Ca_v1.4$  subunits has been reported in mouse cerebral cortex after 7 days of nicotine treatment (Hayashida et al., 2005). A detailed analysis of channel subtype changes showed that at 24 hours after the last of 14 days of nicotine administration to mice, there were reductions in  $Ca_v1.2$  expression in several brain regions, including the VTA and caudate-putamen, but after 7 days of abstinence, there was strong upregulation of  $Ca_v1.2$  mRNA in many brain regions but not the VTA. The  $Ca_v1.3$  mRNA was little changed at either of these intervals (Bernardi et al., 2014). These results suggest that the inconsistencies in the earlier studies were likely due to differences in the time intervals from the last nicotine administration, as well as differences in the nicotine administration schedules.

5. *Clinical Studies on L-Type Calcium Channel Ligands and Nicotine Dependence.* Given the results from the above preclinical studies, there has been surprisingly little clinical study of dihydropyridines in nicotine dependence, at least so far. One clinical trial is in progress, however, involving cue exposure and isradipine for smoking cessation: trial 03083353 on ClinicalTrials.gov. The results are not yet available, but details of the trial protocol have been published (Papini et al., 2020).

#### E. L-Type Calcium Channels and Benzodiazepines and Barbiturates

Evidence for the involvement of L-channels in benzodiazepine and barbiturate dependence is less than for the other drugs of dependence described above because fewer studies have been carried out; some contradictory patterns of changes have been reported.

##### 1. Benzodiazepines and L-Type Calcium Channels.

Some of the acute behavioral effects of benzodiazepines have been reported to be increased by calcium channel antagonists, but other central effects were unaltered or reduced. Acute, but not concurrent, injections of nitrendipine reduced somatic withdrawal signs caused by chronic administration of flurazepam to mice but did not affect the development of tolerance or cause changes in central dihydropyridine-sensitive binding sites (Dolin et al., 1990). A single intraperitoneal dose of nifedipine completely eliminated both anxiety-related and

convulsive behavior in rats during withdrawal from chronic diazepam (Garibova et al., 1998), and in agreement, acute administration of nifedipine prevented anxiety-related withdrawal behavior after cessation of chronic diazepam treatment (El Ganouni et al., 2004). When nifedipine was given concurrently with the diazepam, the somatic withdrawal signs were prevented.

An increase in the number of dihydropyridine-sensitive binding sites was found when bovine chromaffin cells were grown in alprazolam (Brennan and Littleton, 1991). Similarly, prolonged exposure to diazepam, brotizolam, or clobazam increased calcium influx through L-channels, demonstrating a functional change, diltiazem binding, and the expression of  $Ca_v1.2$  and  $Ca_v1.3$ , in primary cultures of mouse cerebral cortical neurons (Katsura et al., 2007). Treatment with flurazepam for 1 week increased high-voltage-activated calcium currents in acutely isolated hippocampal neurons (Xiang et al., 2008).

Few clinical studies have been made in this area. In healthy human volunteers, repeated administration of nimodipine (30 mg three times daily) provided no evidence of interactions between the dihydropyridine and diazepam with regard to pharmacokinetics, safety, or subjective discomfort (Heine et al., 1994).

##### 2. Barbiturates and L-Type Calcium Channels.

Many studies have shown that barbiturates acutely reduce calcium channel conductance, including that through L-channels (Blaustein and Ector, 1975; Heyer and Macdonald, 1982; Earl and Tietz, 2011). When given acutely, nitrendipine did not prevent somatic barbiturate withdrawal signs, but concurrent administration of nitrendipine resulted in prolonged protection against withdrawal hyperexcitability (Rabbani et al., 1994), and administration of nifedipine over 48 hours reduced somatic barbiturate withdrawal signs (Germany and Contreras, 1994). However, in contrast, mixing nifedipine with barbital in food for chronic treatment potentiated the body weight loss and behavioral withdrawal scores after chronic treatment (Suzuki et al., 1995). Concurrent administration of nitrendipine, diltiazem, or nifedipine with a barbiturate had no effect on the development of barbiturate tolerance (Germany and Contreras, 1994; Rabbani et al., 1994). Increased synaptosomal calcium uptake and density of dihydropyridine binding sites were reported in mouse cerebral cortex after chronic barbiturate treatment, and both effects were prevented by concurrent administration of nitrendipine (Rabbani and Little, 1999).

## V. Considerations and Potential Mechanisms

### A. Evaluation of Preclinical Studies

The lack of consideration of effects of the solvents used to dissolve dihydropyridines and the potential consequences of their use in some of the studies



described above have been detailed in section III. B. *Use of Solvents*. Another aspect that complicates evaluation is a frequent lack of information about precisely when the last dose of a chronic drug administration was given and how long after this dose the measurements were made, whether behavioral or neurochemical. It is also essential to state precisely when the L-channel blocker was administered.

In addition, measurements of tissue drug concentrations are rarely carried out, and when they are, brain concentrations are rarely measured, even though these are by far the most important and relevant in this context. Brain levels do not necessarily parallel drug plasma concentrations; clear differences in time courses were shown, for example, between brain and plasma alcohol levels in rats (Nurmi et al., 1999). Measurement of plasma concentrations may therefore be misleading. More pharmacokinetic information is needed in almost all studies; this information is crucial to the understanding of the CNS changes in drug dependence and to the determination of the mechanism(s) of action of potential therapeutic drugs.

Certain other aspects also need to be more widely investigated in the context of potential pharmacological treatments. The contribution of stressful experience, especially early-life stress (Schwandt et al., 2013), needs to be more extensively addressed; links in humans between stress and drug dependence are well established. Imaging and other studies have demonstrated the close similarities between the brain areas involved in relapse drug taking and those affected by stress (Sinha and Li, 2007), and  $Ca_v1.2$  channels have been implicated in the long-term effects of stress (Bavley et al., 2017; Dedic et al., 2018). Stress-induced reinstatement of drug taking is a valuable experimental tool in the study of potential therapies (Bavley et al., 2020).

In humans, acute behavioral and neuronal changes appear after cessation of prolonged intake of a dependence-inducing drug (withdrawal symptoms) and then decline over the next few days or weeks, but prolonged symptoms of craving and other somatic and psychologic withdrawal signs are experienced many months into abstinence. Relapse back into drug taking is the most serious clinical problem and this can occur years later. A number of the animal studies described above have examined the changes that extend long into the abstinence phase. It is notable that effects of an L-channel blocker were seen at 7 and 14 days after the end of repeated nicotine treatment (Bernardi et al., 2014),  $Ca_v1.2$  gene knockout mice exhibited differences in responding for alcohol 4 weeks after alcohol withdrawal (Uhrig et al., 2017), and prior administration of nimodipine prevented

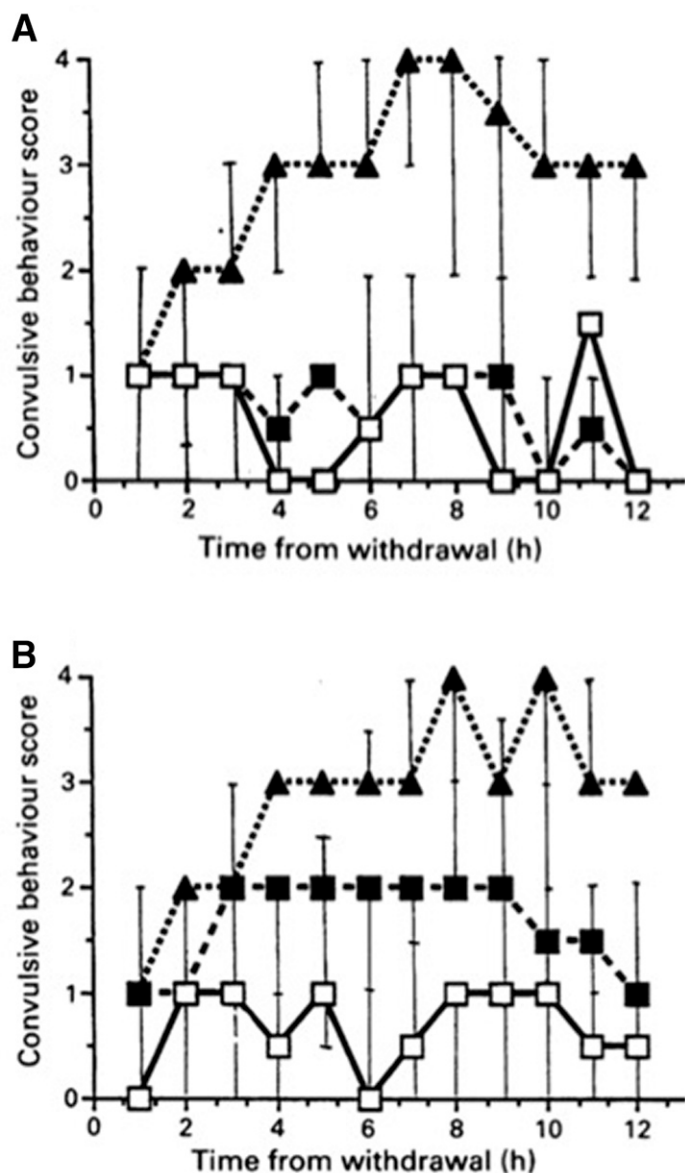
cognitive deficits in rats measured 1 to 2 months into the alcohol abstinence phase (Brooks et al., 2008). More attention has been paid to long-lasting changes in psychostimulant studies, and prolonged alterations in reinstatement of operant self-administration have been demonstrated (see section IV. B. 1a. *Operant self-administration of psychostimulant drugs*). Evidence of increases in L-channels have also been reported at prolonged intervals after cessation of dependence-inducing drugs (Nasif et al., 2005; Ford et al., 2009; Uhrig et al., 2017). These studies indicate that L-channel blockers could have extended therapeutic benefit, but more studies need to be made longer into abstinence phases in dependence.

Another aspect that could usefully be further investigated is effects of L-channel blockers on cue-induced responding, as relapse in humans is often seen in reaction to drug-associated cues (Little et al., 2005; Clemens and Holmes, 2018). Effects of L-channel blockers in adolescent rodents also need to be given more attention, as drug taking in humans commonly begins at this stage. The potentially different actions of L-channel blockers in humans or animals that are drug-dependent, compared with those in normal volunteers or drug-naive animals, are most important; the increases in dihydropyridine binding sites caused by dependence-inducing drugs may have relevance here.

### B. Single or Repeated Administration of L-Type Calcium Channel Ligands

The various effects of L-channel ligands described above are not necessarily all mediated by the same molecular mechanism(s). The acute effects, for example, in protecting against somatic withdrawal signs, and the reductions in the reinforcing effects, appear to be due to the initial direct actions on calcium channels. The former effects have been observed electrophysiologically in vitro as well as in vivo. The effects of longer-term administration of L-channel ligands, however, may be caused by responses or adaptations to the calcium channel blockade that could be downstream from their action on L-channels.

Interpretation of studies involving long-term (chronic) dihydropyridine administration also needs to consider whether the effects seen were due to the continuing presence of the L-channel ligand in the brain or whether they were due to neuronal changes caused by the prior chronic dihydropyridine administration. In the case of tolerance to dependence-inducing drugs, for example, if the amount of L-channel ligand in the brain at the time of testing was sufficient to increase the acute actions of the dependence-inducing drug, this could mask the level of tolerance. The situation is similar with regard to the prevention of withdrawal signs. This is why it is so important that publications state exactly when the last dose of the L-channel ligand dose was given and that studies



**Fig. 1.** The effects of either alcohol alone or alcohol plus nitrendipine given to C57 mice in the drinking fluid for 12 weeks on the severity of withdrawal convulsive behavior after alcohol withdrawal. (A) The nitrendipine was removed from the drinking fluid 24 hours before withdrawal of the alcohol. The prior consumption of nitrendipine abolished the withdrawal syndrome, even though little dihydropyridine remained in the brain at the time of testing. Results are median values ( $\pm$  interquartile ranges) for 7–12 mice. The ratings of convulsive behavior were significantly lower in animals given nitrendipine than those for animals given alcohol alone ( $P = 0.005$ ) and were not significantly different from control values, i.e., from ratings of animals that did not receive any alcohol ( $P > 0.1$ ). All comparisons were made on scores between 3 and 12 hours of the testing period: closed triangles = alcohol plus tween vehicle injections; closed squares = alcohol plus nitrendipine injections; open squares = controls. (B) The effects of either alcohol alone or alcohol plus nitrendipine given by intraperitoneal injection for the last 2 weeks only of the 12-week alcohol-drinking period in C57 mice. There was a significant decrease in the withdrawal ratings ( $P < 0.05$ ) for animals given nitrendipine injections compared with those for animals given alcohol alone. The ratings for mice given alcohol plus nitrendipine were significantly higher ( $P < 0.05$ ) than those of control animals not given alcohol: closed triangles = alcohol alone; closed squares = ethanol plus nitrendipine; open squares = controls. (Reproduced with permission from Whittington et al., 1991.)

determine whether residual dihydropyridine was present in the brain at the testing times.

### C. Do L-Type Calcium Channel Ligands Reverse Established Changes in Drug Dependence?

One aspect of the interactions between L-channel blockers and dependence-inducing drugs is crucial to both the potential importance of the former drugs in the treatment of drug dependence and to understanding of the mechanism(s) involved. This is whether actual reversal of any of the neuronal changes underlying dependence can be brought about. If the calcium channel blockers only ameliorated certain aspect of dependence, such as withdrawal symptoms, then they still might have some therapeutic value. However, given the chronic relapsing nature of drug dependence, such value is likely to be limited, as the desire and craving for drugs would reappear, as happens during the frequent relapses when addicts attempt to stop taking a drug. However, if pharmacological treatments could be found that actually *reversed* some of the underlying CNS changes causing the dependence, and also had *prolonged* action after the treatment stopped, this would have far greater clinical value.

Another question is whether L-channel blockers have effects in reducing dependence when given prior to, or concurrently with, the chronic intake of a dependence-inducing drug, or later when adaptive changes have already developed. Clearly, a compound that was only effective if given prior to initial intake of the dependence-inducing drug would be of little or no value for therapeutic purposes. However, it would be of considerable advantage to have a treatment that reversed brain changes causal to the dependence, whether given either while an addict was still taking a drug or during abstinence periods.

As described in the preceding sections, there is preclinical evidence that such “reversal” effects can be produced by dihydropyridine L-channel blockers. In one study, reduction of the somatic withdrawal signs was apparent when nitrendipine was administered only during the last 2 weeks of a prolonged alcohol administration (Whittington et al., 1991), illustrated in Fig. 1. Parallel experiments demonstrated that, at the beginning of those 2 weeks, sufficient alcohol had been consumed by the rodents to produce somatic withdrawal signs. Withdrawal severity was also reduced even when the last administration of nitrendipine was 48 hours before alcohol withdrawal and the measured brain concentration of nitrendipine was very low. In addition, the electrophysiological signs of withdrawal hyperexcitability in hippocampal slices were not seen after such treatment, and the increase in dihydropyridine binding normally produced by chronic alcohol consumption was no longer significant (Whittington et al., 1991; Bailey et al., 1998b). Similarly, administration of nimodipine to rats at the end of long-term alcohol consumption prevented cognitive

deficits when these were measured 1 to 2 months later, a time interval that far exceeded the clearance of nimodipine (Brooks et al., 2008).

Reversal of established tolerance to opioids was indicated when intracerebroventricular administration of nifedipine partly reduced morphine tolerance in mice that were already tolerant; the nifedipine dose did not alter the actions of morphine in nontolerant animals (Smith et al., 1999a). Prevention of sensitization to alcohol by nicotine, after such sensitization, was established has been demonstrated after administration of nifedipine (Bernardi et al., 2014), as illustrated in Fig. 2.

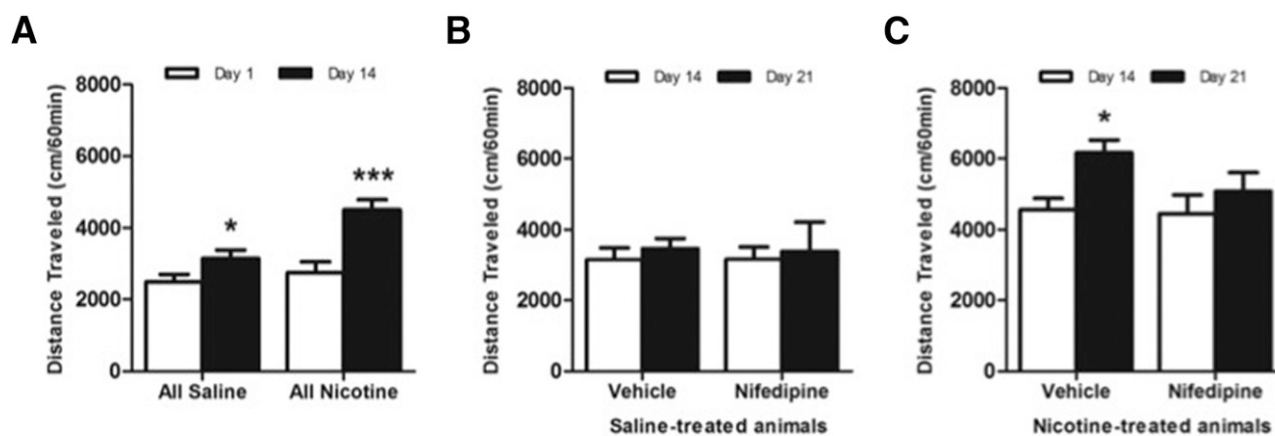
Evidence of reversal effects has particularly been found with psychostimulant drugs. Isradipine abolished previously acquired cocaine conditioned place preference when given prior to reinstatement testing, including responding to cocaine-associated cues (Degoulet et al., 2016). Similar results with alcohol-conditioned place preference were found by these authors. Reinstatement of cocaine-induced place conditioning by either cocaine cues or stressful experience was prevented by isradipine given just prior to the reinstatement (Bavley et al., 2020). Addy et al. (2018) showed that administration of isradipine either systemically, or into the VTA, reduced cocaine seeking in abstinent rats previously trained to self-administer cocaine, as illustrated in Fig. 3. These data suggest that blockade of central L-channels has great potential for the treatment of dependence on a range of drugs.

#### D. Potential Mechanisms for Effects of L-Type Channel Blockers in Drug Dependence

Another major question about the mechanisms of the effects of the L-channel ligands described above

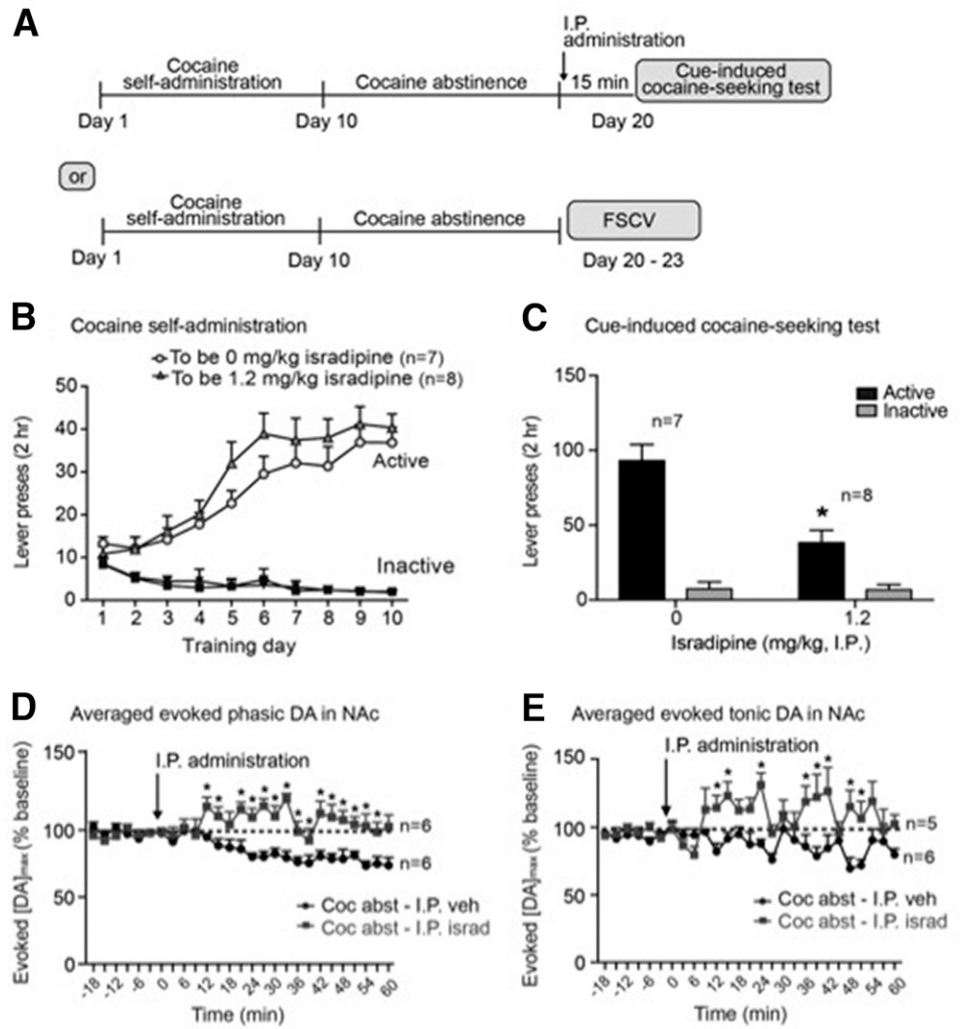
is whether these are actually due to interaction with  $Ca_v1.2$  and/or  $Ca_v1.3$  channels or due to effects at other site(s). Binding of L-channel ligands to dihydropyridine is stereospecific, and when enantiomers were compared in the studies above, both in vivo and in vitro, it was the active stereoisomer that had greater effects. This strongly suggests that interactions described were mediated by dihydropyridine effects on the L-channels. However, owing to the ubiquitous nature of calcium as a second messenger, some of the effects described may in fact be due to mechanisms downstream from the neuronal calcium channels. The paragraphs below describe actions of L-channel blockers that might explain their effects in drug dependence. This is in no way an exhaustive list, as L-type calcium channels are involved in so many aspects of central synaptic transmission.

One likely explanation for the effects of L-channel ligands involves the mesolimbic dopamine system, a brain area known to be involved in reinforcement and in the salience of cues (Wise and Robble, 2020). The salience of environmental and other drug-related cues (i.e., how important they are to an organism) is crucial during drug dependence, as a focus on drug-related stimuli is a notable characteristic of drug dependence. The VTA contains dopaminergic cell bodies that project to many brain areas, in particular the nucleus accumbens and prefrontal cortex, and recent work has demonstrated their crucial importance in reward and aversion (Yuan et al., 2019). All the different groups of dependence-inducing drugs activate transmission in this dopaminergic system. Rats will self-administer alcohol into the VTA (Gatto et al.,



**Fig. 2.** Nifedipine impairs abstinence-induced nicotine locomotor sensitization but has no effect in saline-treated controls. (A) Locomotor activity measured on days 1 and 14 in all mice repeatedly injected with either saline or nicotine (0.175 mg/kg, i.p.) on days 1–14 prior to group assignment for subsequent vehicle of nifedipine treatment. Both saline- and nicotine-treated mice demonstrated a significant increase in locomotor activity from day 1 to day 14 (\* $P < 0.05$ , \*\*\* $P < 0.001$  vs. day 1). (B) Locomotor activity measured on days 14 and 21 in mice injected with saline on days 1–14 and separated into groups that received vehicle or nifedipine (10 mg/kg) treatment on days 15–20. There was no effect of vehicle or nifedipine treatment on locomotor activity on day 21 compared with day 14 in mice that received saline injections on days 1–14 and 21. (C) Locomotor activity measured on days 14 and 21 in mice injected with nicotine (0.175 mg/kg, i.p.) on days 1–14 and separated into groups that received vehicle or nifedipine (10 mg/kg, intraperitoneal) treatment on days 15–20 of the 7-day nicotine abstinence period. Mice treated with vehicle on days 15–20 showed a significant increase in locomotion in response to nicotine (0.175 mg/kg, i.p.) on day 21 compared with day 14, indicative of nicotine-induced sensitization (\* $P < 0.005$  vs. day 14). Mice treated with nifedipine on days 15–20 failed to show the same increase in locomotion in response to nicotine (0.175 mg/kg, i.p.) on day 21 compared with day 14, indicative of an impairment of nicotine-induced sensitization. (Reproduced with permission from Bernardi et al., 2014.)

**Fig. 3.** Systemic isradipine administration attenuates cue-induced cocaine seeking and enhances nucleus accumbens (NAc) dopamine (DA) signaling. (A) Timeline of systemic isradipine experiments for cue-induced cocaine seeking and voltammetric analysis of dopamine signaling in the nucleus accumbens. (B) Days 1–10 of cocaine self-administration training based on “to be” experimental assignments for the cue-induced cocaine-seeking test. (C) Systemic administration of isradipine on abstinence day 10 significantly attenuated cue-induced cocaine seeking. (D) Average evoked phasic dopamine release in the nucleus accumbens of cocaine abstinent rats. Systemic isradipine administration led to significantly greater phasic dopamine release compared with systemic vehicle administration. (E) Averaged evoked tonic dopamine release in the nucleus accumbens of cocaine abstinent rats. Systemic isradipine also led to significantly greater tonic dopamine release compared with systemic vehicle. Data are presented as  $\pm$  S.E.M. \* $P < 0.05$  [(C) post hoc significant decrease in active lever presses for vehicle vs. isradipine rats, Bonferroni corrected comparisons; (D and E) post hoc significant dopamine differences in vehicle vs. isradipine rats, Bonferroni corrected comparisons]. Coc abst, cocaine abstinence; FSCV, in vivo fast scan cyclic voltammetry; israd, isradipine; I.P., intraperitoneal; veh, vehicle. (Reproduced with permission from Addy et al., 2018.)



1994), and chronic administration of cocaine or amphetamine has been associated with alterations in dopaminergic neuroanatomy and transmission (Nicola and Deadwyler, 2000; Onn and Grace, 2000; Schierberl et al., 2011). Marked reductions in spontaneous firing were reported in mouse VTA neurons measured in vitro in slices prepared 24 hours or 6 days after cessation of chronic alcohol treatment, times when behavioral signs of alcohol withdrawal had subsided. Increases in striatal D2-like receptor affinity were also demonstrated at these times and also after 2 months of abstinence (Bailey et al., 1998a, 2001).

L-channels are crucial in the control of mesolimbic transmission. They are involved in the pacemaker mechanisms that control spontaneous firing in VTA dopaminergic neurons, including the burst firing that is activated on exposure to salient stimuli, and are implicated in conditioning (Mercuri et al., 1994; Liu et al., 2014). The  $Ca_v1.3$  subtype in VTA dopaminergic neurons was shown to be important in the regulation of both single-spike and burst firing, whereas

activation of  $Ca_v1.2$  channels may also contribute to the latter (Liu et al., 2014).

L-channels are also involved in the excitability of medium spiny neurons, which make up a large proportion of the cells in the nucleus accumbens. These neurons have bistable membrane potentials, and the transitions between different states involve both  $Ca_v1.2$  and  $Ca_v1.3$  channels (HernandezLopez et al., 1997; Cepeda et al., 1998; Striessnig et al., 2014). Firing in these neurons is controlled by glutamatergic afferents from hippocampus, prefrontal cortex, and amygdala, and these inputs are modulated by dopamine. In the “down” state, dopamine is inhibitory, but in the “up” state, dopamine increases glutamate excitation, which increases dihydropyridine-sensitive calcium channel conductance (Cooper and White, 2000). Dihydropyridine L-channel blockers suppress the transition to the upstate in these neurons and therefore could reduce changes in this pathway during drug dependence (Olson et al., 2005)

L-channels also play a vital role in the excitability of neurons in the prefrontal cortex, an area that is

implicated in decision making and drug dependence (Young and Yang, 2004). Studies in  $Ca_v1.2$ -deficient mice showed recently that in the prelimbic cortex, which projects to the accumbens,  $Ca_v1.2$  channels are necessary for both cocaine-induced and stress-induced reinstatement of cocaine conditioned place preference (Bavley et al., 2020).

The development of drug dependence involves gradual and progressive changes in central nervous system function over time, with resultant alterations both in the effects of the drugs, and in neuronal functions in the absence of the drugs. Wide-ranging studies on protein expression in animals and humans after long-term intake of different types of drugs (alcohol, amphetamine/methamphetamine, cocaine, opioids, or nicotine) have shown some common patterns of protein changes in signaling pathways related to neuronal function (Wang et al., 2011). In neurons, regulation of gene expression by activity-induced increases in calcium is important in long-term neuronal adaptive responses, and much activity-dependent gene expression requires activation of L-channels (Chawla, 2002; Barbado et al., 2009; Puri, 2020). Evidence has been provided for the involvement of microRNAs in transcriptional events concerning genes implicated in addiction (Li and van der Vaart, 2011). Age-specific changes in expression pattern have been demonstrated in the VTA after 2 weeks of nicotine treatment of adult and adolescent rats, and the authors suggested these might reflect the vulnerability of adolescents to addictive drugs (Doura et al., 2010). Actions on these processes could underlie the effects of L-channel ligands during drug dependence, particularly the longer-term consequences of chronic administration.

Synaptic plasticity mechanisms, such as LTP and long-term depression, may be involved in the actions of the L-channel ligands in drug dependence; there are many reports of changes in synaptic plasticity during dependence on drugs. L-channels are involved in transcriptional processes and regulation of calcium-dependent protein kinases and other messengers (Misra et al., 1994; Finkbeiner and Greenberg, 1998; Rajadhyaksha et al., 1999). The elegant review of both intrinsic and synaptic plasticity changes after chronic cocaine administration by Francis et al. (2019) emphasized the importance of L-channels in the VTA. Chronic intermittent alcohol treatment of primary cortical neuronal cultures resulted in alterations in mRNA expression known to be involved in synaptic plasticity (Melendez et al., 2012) and LTP of corticospinal activity in humans changed to long-term depression after administration of nimodipine (Wankerl et al., 2010). Deletions of  $Ca_v1.2$  channels in hippocampus and neocortex resulted in deficits in NMDA-receptor independent synaptic plasticity and

in spatial learning (Kleppisch et al., 2004; Moosmang et al., 2005).

There is considerable information now available about the similarities between the neuronal changes involved in drug dependence and those in memory and learning (Dacher and Nugent, 2011; Robinson and Atkinson, 2013). L-channel blockers can alleviate acute memory deficits due to seizures, neuroinflammation, age, ischemia, or drugs such as scopolamine or alcohol (Hoffmeister et al., 1982; Batuecas et al., 1998; Brooks et al., 2002; Hopp et al., 2015), and L-channels are involved in age-related cognitive deficits (Thibault et al., 2007; Krueger et al., 2017; Moore and Murphy, 2020). Although early publications suggested that the anti-amnesic actions of L-channel ligands were due to vascular effects, they are now established to occur via neuronal L-channels (Thompson et al., 1990; Landfield, 1993; Quevedo et al., 1998).

L-channel blockers have been found to have some neuroprotective actions that may contribute to their beneficial effects, for example, against brain damage caused by alcohol withdrawal (Chandler et al., 1993; Crews et al., 2004). Localized upregulation of L-channels has been reported after various forms of brain injury (Westenbroek et al., 1998). The chronic alcohol-induced increases in brain corticosterone concentrations in rodents, which last long into the abstinence phase (Little et al., 2008), are likely to contribute to neurotoxicity. They were prevented by administration of an L-channel blocker, as were the concurrent memory deficits (Brooks et al., 2008). Other sites at which L-channel ligands could interact with the development of drug dependence include specific neurotransmitter systems; substance P is a possible candidate, as NK1R antagonists bind to L-channels and have been suggested to be of value in drug dependence (Stanford, 2014).

## VI. The Future

### *A. Summary of L-Channel Ligand Interactions in Drug Dependence*

In summary, compounds with specific actions in blocking L-channels have important, and in some cases unique, actions in models of drug dependence. A summary of their effects is shown in tabular form (Table 2). Patterns common to alcohol, opioids, psychostimulant drugs, and nicotine in preclinical studies are 1) that L-channel blockers can reduce the reinforcing effects of dependence-inducing drugs; 2) that L-channel blockers reduce somatic withdrawal signs; 3) that concurrent administration of L-channel blockers during chronic treatment with the dependence-inducing drug reduces or prevents the adaptive changes that result in tolerance or sensitization; and 4) that chronic or repeated administration of the

TABLE 2 Summary of reported effects of L-channel blockers

This table is an overall summary of the reported effects of L-channel blockers that are related to drug dependence, as described in the sections of this review. References and more details of the data can be found in the individual sections above. Note that only a small number of clinical investigations have been carried out so far, and the dose ranges used were necessarily limited by the cardiovascular effects of the L-channel ligands. Very few studies have been made on benzodiazepines or barbiturate.

Dependence-Inducing Drug Group	Reduction of Somatic Withdrawal Signs	Reduction of Reinforcing Effects	Reduction or Prevention of Tolerance or Sensitization	Increased Dihydropyridine Binding after Chronic Treatment	Reduction of Clinical Effects of Dependence-Inducing Drug
Alcohol	Yes	Yes	Yes	Yes	Maybe
Psychostimulants	Yes	Yes	Yes	Yes	Maybe
Opioids	Yes	Yes	Yes	Yes	Maybe
Nicotine	Yes	Yes	Yes	Maybe	?
Benzodiazepines	Yes <sup>a</sup>	?	?	Yes <sup>a</sup>	?
Barbiturates	Yes <sup>a</sup>	?	No	Yes	?

"?" indicates that evidence is not available. "Maybe" indicates conflicting or equivocal results.

<sup>a</sup>One study with conflicting results.

dependence-inducing drug causes upregulation of central dihydropyridine binding sites. The L-channel ligands do not appear to have any consistent reinforcing or rewarding effects of their own, an aspect essential for their potential as novel pharmacological treatments for dependence.

The reinforcing and/or rewarding properties of dependence-inducing drugs are well established and widely recognized to be related to drug dependence. They are therefore extremely important for the development of therapies to reduce relapse and craving in human addicts. L-channel blockers have been demonstrated to reduce the reinforcing effects, whether measured by operant self-administration studies or conditioned place preference, of alcohol, psychostimulants, opiates, and nicotine. Although the methodology used in these studies varies, this pattern of effects of L-channel blockers is consistent.

Another notable aspect is the ability of L-channel blockers to prevent the alterations in the behavioral effects of dependence-inducing drugs seen during their long-term intake. This pattern has been observed whether the repeated intake reduced the dependence-inducing drug's actions (tolerance) or increased them (sensitization). Throughout this review these changes have been called "adaptive," but they could also be described as "reactive," as they represent physiologic reactions of the organism to the prolonged presence of the drug in the CNS. Their significance with regard to drug dependence is still not fully understood, but they frequently accompany dependence, and their prevention by L-channel blockers is important in this context.

The exact role of the increases in dihydropyridine binding in the development of dependence has yet to be determined, but such binding site increases are seen after chronic administration of each of the drug groups that cause dependence, despite the different initial target sites, suggesting that the increased binding has some bearing on the dependence. Accompanying increases in calcium channel conductance have been recorded, indicating that the binding changes have functional relevance. Reports have also

shown that the L-channel increases can last long into abstinence and may therefore have relevance to the prolonged craving and relapse liability seen in humans. Concurrent administration of L-channel blockers has been reported to prevent the increases in binding, as well as the behavioral changes. Detailed identification is under way of calcium channel isoform changes in the areas of the brain that are most important in drug dependence.

Few clinical studies have been carried out so far on L-channel ligands in drug dependence. Some of the trials on the effects of L-channel blockers in individuals dependent on alcohol or opioids suggested beneficial effects, but side effects were problematic. Reports have suggested reductions in the subjective effects of cocaine and methylamphetamine with L-channel ligands, but the data are sparse and not consistent. The primary problem is that the concentrations of currently available dihydropyridines required to block L-channels in central neurons are higher than those that affect channels in peripheral tissues (see section III. *Doses, Concentrations, and Pharmacokinetic Aspects*), and the potential for adverse cardiovascular effects has therefore limited the doses used in clinical trials. This was considered to be a major impediment in a recent review of clinical trials of nimodipine for a range of disorders (Carlson et al., 2020). Insufficient dose level was also stated recently by the Parkinson Study Group STEADY-PD III to be the most likely reason for the negative results in a clinical trial of isradipine in Parkinson disease (Papini et al., 2020; Parkinson Study Group, 2020), and this was highlighted in the journal *Editorial* (Maiti and Perlmutter, 2020). New drugs with more selective actions, described above in section VI. B. *Potential Drug Candidates* could provide a solution to this problem. Another important factor to be considered in clinical studies is that the effects of L-channel ligands may be different in human volunteers who are not drug-dependent from those in participants in trials who have been drug-dependent for some time or have been receiving prolonged therapeutic drug treatment, for example, opioids for chronic pain.

The similarities in the preclinical interactions of L-channel blockers with the four primary groups of dependence-inducing drugs, alcohol, psychostimulants, opioids, and nicotine, described in this review are remarkable. They suggest that L-type calcium channels might be part of an underlying mechanism involved in dependence on these drugs, in addition to—but separate from—the drugs' initial target sites. The concept of a common mechanism involved in dependence on different drug types is not in any way new. Prolonged administration of one type of these drugs, for example, chronic alcohol intake, has been shown to increase sensitization caused by repeated administration of drugs of a totally different type, e.g., psychostimulants or nicotine, when this is measured at long intervals after cessation of the alcohol withdrawal signs (Manley and Little, 1997; Watson and Little, 1999b), but so far, the elements of such a mechanism have been elusive. The L-type calcium channels could potentially be an important component of such a mechanism.

### B. Potential Future Drug Candidates

Very few structure-activity studies, or even comparison of the effects of more than one compound, have been carried out on the actions of L-channel ligands in the drug dependence studies described above, and more are needed. Differences have long been known within the peripheral actions of dihydropyridine compounds: nimodipine, for example, has a greater effect than other dihydropyridines on cerebral blood vessels (Towart et al., 1982), and nisoldipine shows differential interactions with recombinant channels (Morel et al., 1998). In some instances, diltiazem exhibits different interactions with drugs that cause dependence, as well as in its binding to dihydropyridine sites. However, as described in section II. E. *Selectivity of L-Channel Blocking Drugs*, so far no compounds have been developed that are completely selective for neuronal, as opposed to cardiovascular, calcium channels. The importance of these neuronal channels in psychiatric disorders has been recognized only in recent years. There are changes in L-channels with age and certain inherited disease conditions and genetic variations, and the therapeutic use of L-channel blockers has been suggested for Parkinson disease, Alzheimer disease, and bipolar depression. The use of a limited number of dihydropyridines in the treatment of alcohol dependence was patented back in the 1980s and 1990s (EU patent EP0330924B1, US Patents 4,918,076 and 5,665,740), but these patents were registered before the current development of more selective ligands, and these patents have now expired. There is now a great opportunity for the development of more selective compounds for therapeutic treatment, and the development of more selective versions of these drugs has gained a new

priority. The results described in section II. E. *Selectivity of L-Channel Blocking Drugs* above indicate that drug selectivity between channel subtypes and isoforms is possible, and major advances in this direction are likely in the future.

One potentially promising drug has been described that has selective behavioral effects. The compound MEM1003 (also known as Bay K4406) was shown to improve performance in preclinical tests of memory, including the water maze, eye-blink conditioning, attentional set-shifting, and executive function, at doses that did not affect blood pressure (Rose et al., 2007). MEM1003 has undergone clinical trials in other psychiatric disorders (Murray et al., 2007), but this interesting compound does not yet appear to have been examined in the context of dependence on alcohol or other drugs.

Although some dihydropyridine compounds possess mixed agonist and antagonist actions, and have been described as partial agonists (Triggle, 2003), so far no compound has been demonstrated with high-affinity dihydropyridine binding but no intrinsic efficacy, i.e., that neither increases nor decreases L-channel conductance. It is possible that no drug with such a combination of effects can be made because of the nature of the channel complex. However, in the past, the discovery of benzodiazepine ligands that lacked intrinsic activity, and others that possessed inverse agonist activity, led to the two-way receptor theory (Nutt et al., 1982), which completely changed our view of benzodiazepine and other receptor complexes. Since it is clear that drugs binding to dihydropyridine binding sites can have degrees of positive or negative efficacy (Schramm et al., 1983), the possibility of a ligand with little or no intrinsic efficacy remains. This is at present purely speculative (although it has previously been conjectured; R. Towart, personal communication), but it could be an interesting line of approach, as such a compound could—again, speculatively—stabilize particular states of the L-channel complexes and thus prevent or alter the changes caused by dependence-inducing drugs.

The cost to health services of drug dependence, particularly on alcohol and nicotine, is extremely large. Worldwide, alcohol is responsible for over 5% of all deaths and over 13% of deaths for age 20–39 years, and smoking causes over 13% of all deaths over age 30 (World Health Organization data). Licit and illicit drug use are estimated to cost the human population more than a quarter of a billion disability-adjusted life years (Peacock et al., 2018). The benefits in terms of health, physical, psychiatric, and social, of a novel and effective pharmacological treatment of these problems, without intrinsic dependence liability, would be considerable.



### C. Conclusions

The overall conclusions are that there are common patterns in the effects of L-channel ligands in preclinical models of alcohol, opioids, psychostimulant drugs, and nicotine dependence. Specifically, these include reduction of reinforcing effects, as measured by a variety of methods, reduced severity of somatic withdrawal syndromes, and prevention of the development of tolerance and sensitization to the dependence-inducing drug. There is also evidence that the L-channel blockers can reverse previously induced dependence-related physiological changes. This spectrum of effects of the L-channel blockers in drug dependence is unique. When this author began working on the effects of L-channel blockers in alcohol dependence over 30 years ago, these drugs were widely considered to possess only cardiovascular actions, without any effects on the CNS. Now, interest has been aroused in their central actions in the light of new knowledge about the many functions of L-channels in the brain, the diversity of such channels, and their potential roles in CNS pathologic disorders. Now that L-channel blockers with greater selectivity for channel subtypes are being developed, their therapeutic potential is considerable. As more selective channel blockers become available for clinical use, the possible efficacy of such compounds in the treatment of drug dependence needs to be examined.

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