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Differences Between Calcium Channel Inhibitors in Their Effects on Phencyclidine-Induced Behavioral Stimulation in Mice

JACK A. GREBB,[1] KELLEY A. ELLSWORTH AND WILLIAM J. FREED

Neuropsychiatry Branch, National Institute of Mental Health, Saint Elizabeths Hospital Washington, DC 20032

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GREBB, J. A., K. A. ELLSWORTH AND W. J. FREED. *Differences between calcium channel inhibitors in their effects on phencyclidine-induced behavioral stimulation in mice.* PHARMACOL BIOCHEM BEHAV **23**(4) 613–618, 1985.—Sixteen calcium channel inhibitors (CCI's) were tested in a model utilizing phencyclidine (PCP)-induced behavioral stimulation in mice. There were marked differences in the effects of CCI's both within subclasses and between subclasses of CCI's. All of the dihydropyridines and possibly flunarizine were effective in blocking PCP-induced behavioral stimulation. Papaverine derivatives, including verapamil, and several other CCI's, were ineffective.

Calcium channel inhibitors Phencyclidine Mice

RECENT reviews on the calcium channel inhibitors (CCI's) have emphasized two major points. First, the CCI's have a wide range of structures (see Fig. 1) as well as a variety of mechanisms of action. Second, calcium channels themselves are heterogeneous, and different tissues have varying types of calcium channels which diverge in their responses to the same CCI's [16]. Although many comparative studies of the CCI's in single cardiologic models have been reported, there is very little comparative data of multiple CCI's in a single model of relevance to CNS pharmacology. Therefore, to investigate potential neuropsychiatric applications of the CCI's, we have compared the effects of 16 different CCI's on phencyclidine (PCP)-induced behavioral stimulation in mice.

Binding studies have suggested a neuropharmacological role for the CCI's. Specific binding sites for the dihydropyridine-CCI's occur in both rat [30] and human brain [9]. In the rat brain this binding is concentrated in the hippocampus, caudate, and cerebral cortex [24]. Whether the dihydropyridine binding sites are, in fact, calcium channels themselves has yet to be demonstrated [27]. *In vitro* studies have shown that verapamil blocks and reverses phencyclidine (PCP)-induced vasospasm in isolated canine cerebral arteries with a concentration range of PCP relevant to its clinical toxicity [2]. Verapamil and gallopamil are potent displacers of PCP binding in rat brain sections [31], and a variety of CCI's have differential abilities to displace PCP binding to *Torpedo* electric organ membrane [11]. Verapamil also inhibits amphetamine-induced stimulation of catecholamine synthesis in rat striatum *in vitro* [38].

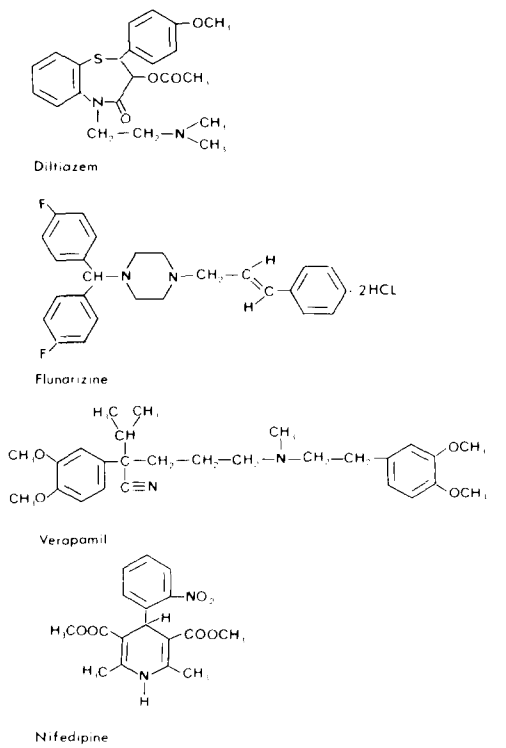

FIG. 1. Structures of representative calcium channel inhibitors.

[1]Requests for reprints should be addressed to Dr. Jack A. Grebb, NYU Medical Center, Department of Psychiatry, 550 First Avenue, New York, NY 10016.

TABLE 1

BLOCKADE OF PCP-INDUCED STIMULATION BY ORGANIC CALCIUM INHIBITORS[a]

Drug	Dose (mg/kg)	N	Activity Before[b]	Stimulation Ratio[c]
Vehicle	—	34	333 ± 50.6	2.14 ± 0.20
Benzothiazepine-derivative:				
Diltiazem	50	8	67 ± 12.4§	5.76 ± 96§
Dihydropyridine-derivatives:				
Nifedipine	50	11	345 ± 69.4	0.29 ± 0.11†
Nimodipine	50	12	191 ± 41.4*	0.66 ± 0.20*
Nisoldipine	12.5	4	137 ± 70.4†	0.49 ± 0.19†
Nitrendipine	50	12	99 ± 17.0‡	0.10 ± 0.04‡
PY 108-068	50	11	370 ± 76.9	0.22 ± 0.09†
Piperazine-derivatives:				
Gallopamil	25	5	31 ± 7.0§	3.94 ± 1.26†
Tiapamil	50	5	218 ± 63.3	3.05 ± 0.50
Verapamil	50	8	67 ± 7.7§	4.01 ± 0.85†
Piperazine-derivatives:				
Cinnarizine	50	6	223 ± 48.3	1.68 ± 0.49
Flunarzine	50	14	162 ± 40.5*	0.91 ± 0.16
Lidoflazine	50	6	456 ± 72.4	1.73 ± 0.26
Others:				
Fendiline	50	6	117 ± 27.2†	2.60 ± 0.70
Molsidomine	50	6	499 ± 119.8	1.49 ± 0.63
Perhexiline	50	5	78 ± 15.7†	3.48 ± 0.70
Prenylamine	50	11	108 ± 25.9†	3.89 ± 0.78†

[a] Data are shown as mean ± SEM.

[b] Activity before PCP, after administration of CCI. Units = counts per 30 minutes.

[c] Stimulation ratios computed as activity during 30 minutes after PCP administration divided by the activity during 30 minutes before PCP administration.

Statistically different from vehicle alone: * $p<0.10$; † $p<0.05$; ‡ $p<0.01$; § $p<0.005$.

Behavioral studies in animals have demonstrated further effects of the CCI's. The dihydropyridines have been reported to (1) block PCP-induced, but not apomorphine-induced, stereotypy in the rat (nisoldipine) [34]; (2) increase electroencephalographic activity in the neocortex and limbic areas of the rabbit (nimodipine) [19]; and (3) block the sleep-induction effect, but not the anticonvulsant or anxiolytic properties of the benzodiazepines in the rat (nifedipine) [26]. In an extensive battery of neuro- and psychopharmacologic tests another dihydropyridine, nimodipine, blocked pentetrazol-induced seizures and caused impaired motor coordination [18]. Verapamil was found to block amphetamine-induced circling behavior in mice with 6-hydroxy-dopamine-induced striatal lesions [15].

Three double-blind, placebo-controlled studies in humans have been reported. Verapamil has been found to reduce manic symptoms in three patients [8]. A dihydropyridine has been reported to improve neuropsychologic test performance in 13 mildly to moderately demented patients [5]. Finally, flunarizine was effective in reducing seizure activity in 10 therapy-resistant children [29]. The possible use of the CCI's in affective disorders is suggested also by the report of a 53-year-old woman whose depression improved with verapamil [20]. This potential application is further supported by the report of mania as a side-effect of diltiazem in one patient [4]. With regard to movement disorders, diltiazem was reported to cause akathisia in one patient [21], and the CCI's have been suggested as a possible therapeutic agent for tardive dyskinesia and other movement disorders [17,23]. A practical advantage which would accrue from the use of CCI's for neuropsychiatric conditions is the relatively low incidence and severity of side effects, at least compared with many currently used psychotropic agents [22].

This present study, therefore, further investigates the CCI's as potential neuropharmacologic agents. The design extends previous investigations of the effects of CCI's on PCP, and allows the comparison of multiple CCI's in a single model.

METHOD

A total of 190 adult inbred female Swiss-Webster mice were housed in groups of 4–8 on a 12-hour light-dark cycle, and allowed free access to food and water. Each mouse was used only once.

Phencyclidine HCl (1-(1-phenylcyclohexyl)piperidine HCl), obtained from Phillips-Roxane, Inc., Saint Josephs, MI, was dissolved in normal saline in a concentration such that a dose of 5.0 mg/kg was administered in a volume of 10 ml/kg by intraperitoneal (IP) injection. In a previous study [13] this dose was found to produce 67% of the maximal degree of stimulation. CCI's were obtained from a variety of sources (see acknowledgements) and drug solutions were made just prior to use. CCI's were administered IP in a

TABLE 2
EFFECTS OF CCI'S (50 MG/KG) ON ACTIVITY OF INBRED MICE

	Dihydropyridines	Piperazines	Papaverines	Others
Reduced	Nitrendipine Nisoldipine* Nimodipine	Flunarizine	Gallopamil† Verapamil	Diltiazem Fendiline Perhexiline Prenylamine
No change from vehicle alone	Nifedipine PY 108-068	Cinnarizine Lidoflazine	Tiapamil	
Increased				Molsidomine

TABLE 3
EFFECTS OF CCI'S (50 MG/KG) ON STIMULATION RATIOS IN PCP-STIMULATED MICE

	Dihydropyridines	Piperazines	Papaverines	Others
Reduced	Nifedipine Nimodipine ($p = 0.07$) Nisoldipine Nitrendipine PY 108-068	Flunarizine ($p = 0.13$)		
No change from vehicle alone		Cinnarizine Lidoflazine	Tiapamil	Fendiline Molsidomine Prehexiline
Increased			Gallopamil† Verapamil	Diltiazem Prenylamine

* 12.5 mg/kg.
† 25.0 mg/kg.

vehicle of 10% Tween 80 in saline in a volume of 10 ml/kg.

The behavioral activity of each animal was measured in cylindrical Polystyrene jars, 23 cm high, with a 16.3 cm inside diameter. These were placed on eight similar Motron-Produkter Co. activity meter units, using the horizontal photocell banks only. The apparatus was illuminated from above by 50 W lights covered by Kodak No. 1A Safelight filters.

The procedure followed exactly that described previously [13]. To summarize, each mouse was given either a CCI in vehicle, or vehicle alone, and activity was then measured for 30 min. The activity meters were then turned off for five minutes, during which time each mouse was given 5 mg/kg of PCP. The activity was then measured for a second 30 min period.

Activity counts were recorded every 10 min for the first and second 30-min periods of activity testing, and were examined in terms of (1) total activity during the first 30-min period, a measure of the effects of the CCI's in the absence of PCP and (2) the ratio of the total activity during the second 30-min period to the total activity during the first 30-min period (stimulation ratio), which was considered to be a measure of the degree of stimulation produced by the PCP. Data were analyzed statistically be a one-way analysis of variance followed by Scheffe multiple comparisons. The null hypothesis was rejected at the 0.05 level.

RESULTS

Table 1 shows the activity before administration of PCP but after the CCI was administered and the stimulation ratios (SR's) for each drug. Data for gallopamil 50 mg/kg, nisoldipine 50 mg/kg and 25 mg/kg are not given because these dosages resulted in the death of virtually all of the mice tested in the first 30 minutes. All of the dihydropyridines were effective in inhibiting PCP-induced locomotor stimulation, with the possible exception of nimodipine ($p = 0.07$). None of the other drugs were effective, although flunarizine showed a trend toward decreasing the SR. A number of the CCI's significantly reduced the activity of the mice in the first 30 minutes; however, these include CCI's with higher SR's (e.g., diltiazem 50 mg/kg), lower SR's (e.g., nimodipine 50 mg/kg), and SR's not statistically different from that for vehicle alone (e.g., perhexiline 50 mg/kg). Molsidomine is unique in showing a statistical trend ($p = 0.06$) toward increasing the activity before the administration of PCP.

Table 2 summarizes the effects of CCI's (50 mg/kg) on the baseline activity of the mice. It is clear that there are varying effects on this measure within a single class of CCI's. Table 3 summarizes the effects of CCI's (50 mg/kg) on the SR. The dihydropyridines are clearly the most effective class. Figure 2 illustrates the effect of one dihydropyridine, PY 108-068 (50 mg/kg), as compared to ve-

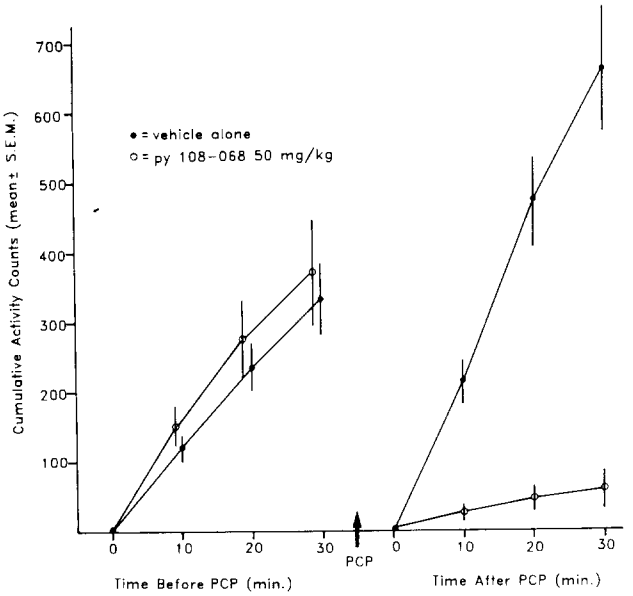

FIG. 2. Effects of PY 108-068 on PCP-induced behavioral stimulation in mice. Cumulative activity counts are shown for the 30 min prior to administration of PCP and for 30 min after PCP administration.

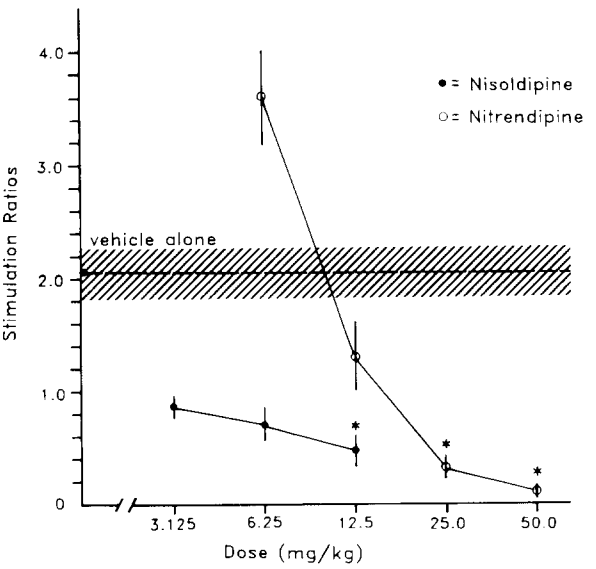

FIG. 3. Dose-effect curves for nisoldipine and nitrendipine. Stimulation ratios (means ± SEM) are shown for varying doses of nisoldipine and nitrendipine.

hicle. PY 108-068 had no significant effect on the pre-PCP activity; however, it significantly reduced the locomotor stimulant effect of PCP. In fact, the PY 108-068-treated animals demonstrated decreased activity in the second 30-min period, the normal habituation response of non-drug-treated animals when returned to a familiar environment. In Fig. 3 a comparison of the effects of two different dihydropyridines in various dosages on the SR is shown.

DISCUSSION

The data presented are consistant with the presence of differential effects both within and between subclasses of CCI's in this model of PCP-induced stimulation. However, there is one important limiting point in the interpretation of these data. Since the CCI's were administered IP, all CCI's cannot be assumed to have crossed the blood brain barrier to an equal degree. Differences between CCI's in this study may therefore be secondary to different CNS concentrations. Also, variable drug-drug interactions between the CCI's and PCP affecting the bioavailability of PCP can not be ruled out. Nonetheless, it can be stated that some of the CCI's did, in fact, block the PCP-induced stimulation.

The most consistent finding of interest is that all the dihyropyridines reduced the SR for the PCP-treated mice. With the possible exception of flunarizine, the dihydropyridines were the only compounds with the ability to reduce the SR's. Dihydropyridine binding sites in the brain are also more specific and more numerous than binding sites in the brain for other CCI's [30].

Flunarizine showed a trend toward reducing the SR. This is in sharp distinction to the other two drugs in this subclass, cinnarizine and lidoflazine. There has been a previous report that it may be more potent in affecting CNS tissue than verapamil [7]. Flunarizine also protects against metrazol-induced [6] and kindled [39] seizures, and allows recovery of normal cerebral electrical activity in curarized rats [37].

The papaverine-derivatives (verapamil, tiapamil, gallopamil) were ineffective in reducing the SR, and even increased it in some cases. This is consistent with an extensive study of tiapamil which showed no CNS activity in behavioral tests [10]. Verapamil, on the other hand, has been reported to have a pentobarbital-like activity on cGMP [36] and to counteract some aspects of hippocampal spike formation *in vitro* [33]. The papaverine binding site may allosterically interact with the dihydropyridine binding site such that it decreases the affinity of the receptor for the dihydropyridines [35]. Two other characteristics differentiate these two subclasses of CCI's. First, whereas the calcium channel inhibition of the dihydropyridines in cardiac tissue is independent of frequency of stimulation of the particular cell, the calcium inhibition of the papaverine-derivatives is frequency-dependent. Second, the papaverines appear to have more effects on other receptor systems, including cholinergic, dopaminergic, noradrenergic, serotonergic, and opiate receptors [12].

Diltiazem increased the SR. The calcium channel inhibition of diltiazem is frequency-dependent, and it is possible that this shared characteristic with the papaverines contributes to their similar effect of increasing the SR's. Diltiazem differs from the papaverines in that its binding seems to allosterically enhance the binding of the dihydropyridines [35].

In a separate study (unpublished data) we examined the effects of CCI's on amphetamine-induced behavioral stimulation. Nifedipine and flunarizine significantly reduced the SR's, and PY 108-068 also showed a trend toward reducing the SR. Nimodipine, nisoldipine, nitrendipine, and all of the papaverines were ineffective. This data further supports differential effects of CCI's in animal behavior.

Animal PCP models may be most relevant to the problem of toxicity in humans who consume these drugs; however, PCP may also provide a model for schizophrenia and other psychoses [1]. The mechanism for the effects of PCP is

quite controversial but undoubtedly quite complex. Previous studies indicate that the effects of PCP are not modulated through a single neurotransmitter system [13], and that at least an interaction with both the dopaminergic and serotoninergic systems is possible [14,25]. An interesting study by Blaustein and Ickowicz [3] reported that PCP blocked potassium channels. These authors hypothesized that the blocking of these channels could, by prolonging action-potential duration in presynaptic nerve terminals, enhance calcium entry and neurotransmitter release. If this hypothesis is correct, the CCI's might have blocked the effects of PCP simply through their inhibition of calcium channels.

The most direct implications of this study may be for the treatment of PCP intoxication clinically. Both acute [1] and chronic [28,32] PCP use can lead to severe psychiatric problems. Although several pharmacological treatments have been suggested, none is generally thought to be effective. Although it is not possible to recommend human application based on this experiment alone, the CCI's may eventually prove to be useful as either primary or adjunctive pharmacotherapy in PCP toxicity. The clinical problems of hypertension and tachycardia with PCP abuse might also respond favorably to the CCI's. The results of this study are also encouraging for additional research on the application of calcium channel inhibitors to other neuropsychiatric disorders such as movement disorders, schizophrenia, and dementia.

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REFERENCES

1. Allen, R. M. and S. J. Young. Phencyclidine-induced psychosis. *Am J Psychiatry* **135:** 1081, 1978.
2. Altura, B. T. and B. M. Altura. Phencyclidine, lysergic acid diethylamide and mescaline: cerebral artery spasms and hallucinogenic activity. *Science* **212:** 1051, 1981.
3. Blaustein, M. P. and R. K. Ickowicz. Phencyclidine in nanomolar concentrations binds to synoptosomes and blocks certain potassium channels. *Proc Natl Acad Sci USA* **80:** 3855, 1983.
4. Bink, D. D. Diltiazem and hyperactivity. *Ann Intern Med* **100:** 459, 1984.
5. Cucinotta, D., M. Mancini, D. Dall'Asta and M. Passeri. Therapeutical manipulation of the age-related change in cerebral functioning with a new 1,4-dihydropyridine derivative. *Eur Neurol* **22:** Suppl 2, 43, 1983.
6. Desmedt, L. K. C., G. J. E. Niemegeers and P. A. J. Janssen. Anticonvulsive properties of cinnarizine and flunarizine in rats and mice. *Arzneimittelforsch* **25:** 1408, 1975.
7. Dubinsky, B., J. N. Sierchio, D. E. Temple and D. M. Ritchie. Flunarizine and verapamil: effects on central nervous system and peripheral consequences of cytotoxic hypoxia in rats. *Life Sci* **34:** 1299, 1984.
8. Dubovsky, S. L. and R. D. Franks. Intracellular calcium ions in affective disorders: a review and an hypothesis. *Biol Psychiatry* **18:** 781, 1983.
9. Ehlert, F. J., W. R. Roeske, E. Itoga and H. I. Yamamura. The binding of ^{3}H-nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex, and ileum of rats. *Life Sci* **30:** 2191, 1982.
10. Eigenmann, R., M. Gerold, F. Hefti, D. Javanovic and G. Haeusler. Tiapamil, a new calcium antagonist, 2. Further pharmacological characterization and toxicology. *Arzneimittelforsch* **31:** 1401, 1981.
11. Epstein, P. M., J. J. Lambert, J. Moraski, Jr. and E. G. Henderson. Calcium channel antagonists displace ^{3}H-phencyclidine binding to *torpedo* electric organ membrane. *Soc Neurosci Abstr* **9:** 213.11, 1983.
12. Fairhurst, A. S., M. L. Whittaker and F. J. Ehlert. Interactions of D600 (methoxyverapamil) and local anesthetics with rat brain alpha-adrenergic and muscarinic receptors. *Biochem Pharmacol* **29:** 155, 1980.
13. Freed, W. J., D. R. Weinberger and R. J. Wyatt. Neuropharmacological studies of phencyclidine (PCP)-induced behavioral stimulation in mice. *Psychopharmacology (Berlin)* **71:** 291, 1980.
14. Freed, W. J., L. A. Bing and R. J. Wyatt. Effects of neuroleptics on phencyclidine (PCP)-induced locomotor stimulation in mice. *Neuropharmacology* **23:** 175, 1984.
15. Fung, Y. K. and N. J. Uretsky. The importance of calcium in amphetamine-induced turning behavior in mice with unilateral nigro-striatal lesions. *Neuropharmacology* **19:** 555, 1980.
16. Glossman, H. and D. R. Ferry. Molecular approach to the calcium channel. *Drug Dev Eval* **9:** 63, 1982.
17. Goldstein, J. A. Calcium and neurotransmission. *Biol Psychiatry* **19:** 465, 1984.
18. Hoffmeister, F., U. Benz, A. Keise, H. P. Krause and V. Neuser. Behavioral effect of nimodipine in animals. Arzneimittelforsch **32:** 347, 1982.
19. Horibe, M., S. Taira, M. Kikuta, K. Aizawa, H. Yamada, M. Nakano, M. Hayashi and T. Shibuya. EEG activating effects of a new vasodilating agent: Nimodipine on the neocortex and limbic EEG activity. *Jpn J Pharmacol* **31:** 269, 1981.
20. Hoschl, C. Verapamil for depression? *Am J Psychiatry* **140:** 1100, 1983.
21. Jacobs, M. B. Diltiazem and akathisia. *Ann Intern Med* **99:** 794, 1983.
22. Lewis, J. G. Adverse reactions to calcium antagonists. *Drugs* **25:** 196, 1983.
23. Lewis, H. A. and N. M. Bacher. Verapamil and depression. *Am J Psychiatry* **141:** 613, 1984.
24. Marangos, P. J., J. Patel, C. Miller and A. M. Martino. Specific calcium antagonist binding sites in brain. *Life Sci* **31:** 1575, 1982.
25. Martin, J. R., M. H. Berman, I. Krewsun, F. S. Small. Phencyclidine-induced stereotyped behavior and serotonergic syndrome in the rat. *Life Sci* **24:** 1699, 1979.
26. Mendelson, W. B., C. Owen, P. Skolnick, S. M. Paul, J. V. Martin, G. Ko and R. Wagner. Nifedipine blocks sleep induction by flunazepam in the rat. *Sleep* **7:** 64, 1984.
27. Miller, R. J. and S. B. Freedman. Are dihydropyridine binding sites voltage sensitive calcium channels? *Life Sci* **34:** 1205, 1984.
28. Misra, A. L., R. B. Pontani and J. Bartolemea. Persistance of phencyclidine (PCP) and metabolites in brain and adipose tissue and implications for long-lasting behavioral effects. *Res Commun Chem Pathol Pharmacol* **24:** 431, 1979.
29. Overweg, J., C. D. Binnie, J. W. A. Meijer, H. Meinardi, S. T. M. Nuijten, S. Schmaltz and A. Wauquier. Double-blind placebo-controlled trial of flunarizine as add-on therapy in epilepsy. *Epilepsia* **25:** 217, 1984.
30. Peroutka, S. J. and G. S. Allen. Calcium channel antagonist binding sites labeled by ^{3}H-nimodipine in human brain. *J Neurosurg* **59:** 933, 1983.
31. Quirion, R. and C. B. Pert. Certain calcium antagonists are potent displacers of ^{3}H-phencyclidine (PCP) binding in rat brain. *Eur J Pharmacol* **83:** 155, 1982.

32. Rainey, J. M. and M. K. Crowder. Prolonged psychosis attributed to phencyclidine: report of three cases. *Am J Psychiatry* **132:** 1076, 1975.
33. Sastry, B. R., S. S. Chirwa, J. W. Goh, H. Maretic and M. M. Pandanaboina. Verapamil counteracts depression but not long-lasting potentiation of the hippocampal population spike. *Life Sci* **34:** 1075, 1984.
34. Shah, A. B., R. M. Poiletman and N. S. Shah. The influence of nisoldipine—a "calcium entry blocker" on drug-induced stereotyped behavior in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **7:** 165, 1983.
35. Synder, S. H. Drug and neurotransmitter receptors in the brain. *Life Sci* **224:** 22, 1984.
36. Szot, P., W. E. Johnson and R. A. Dodson. Effects of verapamil with calcium chloride and calcium chloride plus A 23187 in rat cerebellar levels of cyclic GMP. *Proc West Pharmacol Soc* **25:** 349, 1982.
37. Tolu, E., O. Mameli, M. A. Caria, F. Melis, V. Anania and M. S. Desole. Protective effects of Ca^{++}-antagonist agents on the cerebral electrical activity during anoxia in the rat. *Pharmacol Res Commun* **15:** 833, 1983.
38. Uretsky, N. J., L. Kamal and S. R. Snodgrass. Effect of divalent cations on the amphetamine-induced stimulation of ^{3}H-catechol synthesis in the striatum. *J Neurochem* **32:** 951, 1979.
39. Wauquier, A., D. Ashton and W. Melis. Behavioral analysis of amygdaloid kindling in beagle dogs and the effect of clonazepam, diazepam, phenobarbitone, diphenylhydantoin, and flunarizine on seizure manifestation. *Exp Neurol* **64:** 579, 1979.