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Interactions between phencyclidine and nifedipine at $^{45}Ca^{2+}$-uptake sites on mouse brain neurons

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The effects of phencyclidine (PCP*) on neuronal calcium channels have not been fully evaluated, while in peripheral tissues they remain equivocal. Both blockade and stimulation of calcium channels have been invoked as mechanisms to explain the pharmacologic effects of PCP in cardiac and vascular smooth muscles [1, 2]. The ability of PCP to both enhance and block neurotransmitter release [3] also suggests that facilitation and inhibition of neuronal calcium currents by PCP may exist at the level of the neuron. However, all such studies are complicated by the potent inhibitory effects of PCP on sodium, potassium and *N*-methyl-D-aspartate (NMDA) gated channels [4].

Dihydropyridine (DHP) calcium antagonists interact with specific neuronal binding sites that regulate functionally relevant 'L'-type voltage-dependent calcium channels [5]. We have demonstrated previously that PCP and the acylating derivatives 1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine (METAPHIT, a specific acylator of neuronal PCP binding sites [6]) and 4-isothiocyanato-1-[1-phenylcyclohexyl] piperidine (FOURPHIT) produce specific irreversible changes in neuronal DHP binding sites by interacting with them both directly and in an allosteric manner [7].

We have extended the study of the effects of PCP on voltage-dependent calcium channels by investigating the effects of PCP, FOURPHIT and METAPHIT on K^+-stimulated $^{45}Ca^{2+}$-uptake into a preparation of mouse brain neurons in the absence and presence of the DHP calcium antagonist nifedipine.

Methods

$^{45}CaCl_2$ (1 mCi) was obtained from New England Nuclear-Dupont, Boston, MA. PCP was obtained from the Bureau of Dangerous Drugs, Department of Health and Welfare, Ottawa, Canada. FOURPHIT (hydrochloride salt) and METAPHIT (hydrochloride salt) were synthesized and supplied by Dr. R. Lessor, Department of Chemistry, NIDDK, NIH, Bethesda, MD. Mesh screen (Nitex 210 and 130) were purchased from Tetko, Elmsford, NY. All other reagents were obtained from standard commercial sources and were of the highest purity possible.

Male mice (18–22 g, Charles River, St. Constant, Quebec) were used for all studies. Mouse brain neurons were prepared according to the method used by Skattebol and Triggle [8] for preparation of rat brain neurons. Briefly, mice were killed by cervical dislocation. The brain was rapidly removed and placed into an ice-cold physiologic solution of the following composition (mM): NaCl, 138; KCl, 5.4; Na_2HPO_4, 0.17; KH_2PO_4, 0.22; glucose, 5.5; sucrose, 46.9; and PMSF, 0.1; the pH was adjusted to 7.35 with 1 M NaOH. The brains were then first sieved through a 210 μM mesh screen (Nitex 210) and subsequently twice through a finer 130 μM mesh screen (Nitex 130). All subsequent steps were carried out as described by Skattebol and Triggle [8]. Mouse brain neurons prepared in this manner displayed a heterogenous morphology with many rounded cellular-like structures dispersed between larger aggregates of neuronal tissue. The viability of all components identified as measured using trypan blue was 80–90%. Protein was determined using the modified Lowry method of Miller [9].

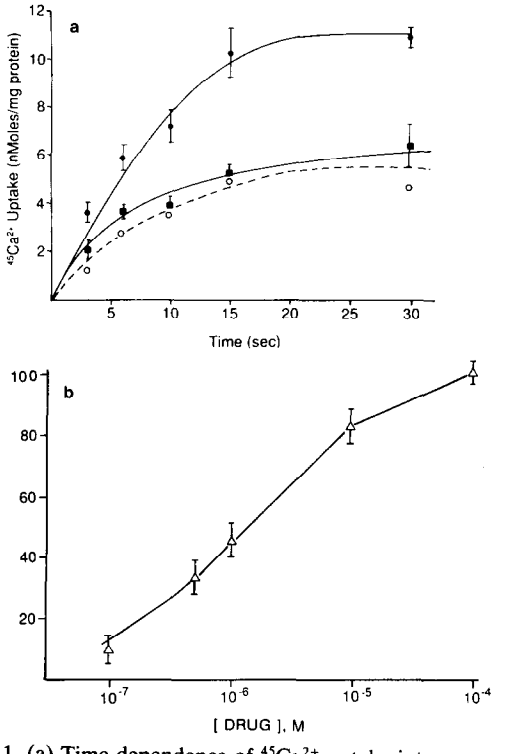

Fig. 1. (a) Time dependence of $^{45}Ca^{2+}$ uptake into mouse brain neurons. Stimulated (●), resting (■) and net (○) $^{45}Ca^{2+}$ uptake in response to 53 mM K^+ are illustrated. Each data point is the mean ± SE of three experiments. The curves through the points were hand drawn. Concentration dependence for PCP inhibition of $^{45}Ca^{2+}$ uptake into mouse brain neurons. Each data point is the mean ± SE of three to six experiments. Net $^{45}Ca^{2+}$ uptake: 2.81 ± 0.3 nmol/mg protein.

* Abbreviations: DHP, dihydropyridine; PCP, phencyclidine; FOURPHIT, 4-isothiocyanato-1-[1-phenylcyclohexyl] piperidine; METAPHIT, 1-[1-(3-isothiocyanatophenyl)cyclohexyl] piperidine; NMDA, *N*-methyl-D-aspartate; and PMSF, phenylmethylsulfonyl fluoride.

Results and Discussion

Net (depolarization-dependent) K^+-stimulated $^{45}Ca^{2+}$ uptake into a preparation of mouse brain neurons was rapid with a mean half-time of 6 ± 1 sec and a maximum uptake of 5.5 ± 1.2 nmol/mg protein reached between 10 and 15 sec (Fig. 1a); values were determined by nonlinear regression analysis using a BMPDAR statistical package (BMDP Statistical Software, Los Angeles, CA) made adaptable to a personal computer. The rapid phase of net $^{45}Ca^{2+}$ uptake was considered to occur between 2 and 5 sec, this period largely representing net $^{45}Ca^{2+}$ entry into neurons through voltage-dependent calcium channels [8]. For this reason, the effects of drugs were measured at 5 sec to assess their actions on voltage-dependent calcium channels. PCP produced a concentration-dependent inhibition of net $^{45}Ca^{2+}$ uptake with an IC_{50} of 4.0 ± 0.2 μM (Fig. 1b); the IC_{50} value was obtained by nonlinear regression analysis. The effect of PCP was reversible since preincubation of mouse brain neurons with 100 μM PCP followed by washing (see Table 1 legend) restored net $^{45}Ca^{2+}$ uptake (Table 1). None of the concentrations of PCP investigated affected resting $^{45}Ca^{2+}$ uptake. FOURPHIT and METAPHIT treatment of mouse brain neurons did not affect significantly net $^{45}Ca^{2+}$ uptake when employed at a concentration of 10 μM (Table 1). However, higher concentrations (100 μM) of FOURPHIT and METAPHIT produced significant decreases (25–40%) in K^+-stimulated $^{45}Ca^{2+}$ uptake (Table 1). Neither FOURPHIT nor METAPHIT affected resting $^{45}Ca^{2+}$ uptake. Nifedipine (10 μM) did not affect net $^{45}Ca^{2+}$ uptake into mouse brain neurons (Table 1). Similar results were obtained in neurons treated with 10 μM METAPHIT. In contrast, neurons treated with 10 μM FOURPHIT responded to nifedipine with a significant inhibition (33%) of K^+-stimulated $^{45}Ca^{2+}$ uptake.

This study clearly demonstrates that PCP was a potent reversible blocker of voltage-dependent calcium channels. The neuronal calcium channel blocking properties of PCP occurred at concentrations below or equivalent to those at which PCP blocks other ion channels. Prior studies in our laboratory, also in mouse brain neurons, demonstrated that the potent NMDA-receptor ion channel antagonist MK-801 did not inhibit $^{45}Ca^{2+}$ uptake, while a series of local anesthetics did, most notably dibucaine and proadefin (Bolger GT, unpublished observations). Thus, the neuronal calcium channel blocking properties of PCP are not due to its NMDA-receptor ion channel antagonist properties, but may be linked to its membrane destabilizing properties [4].

The inability of METAPHIT and FOURPHIT, acylators of PCP and DHP receptors respectively, to inhibit $^{45}Ca^{2+}$ uptake when employed at a concentration of 10 μM suggests that PCP receptors and DHP-linked allosteric PCP binding sites do not mediate the inhibition by PCP of $^{45}Ca^{2+}$ uptake. The inhibition of $^{45}Ca^{2+}$ uptake at higher concentrations of METAPHIT and FOURPHIT may be due to a direct acylation of the calcium channel pore.

In both the rat hippocampal slice and cerebellar Purkinje cell preparations METAPHIT has been shown to antagonize the actions of PCP [10]. Behaviorally, METAPHIT either possessed persistent agonist-like activity [11] or produced a functional antagonism of PCP-induced locomotor activity through presynaptic mechanisms unrelated to a specific blockade of PCP receptors [12]. Thus, the complex behavioral and electrophysiological effects of METAPHIT may arise from interactions with other sites, possibly voltage-dependent calcium channels.

The finding that FOURPHIT (10 μM) but not METAPHIT (10 μM) treatment revealed a $^{45}Ca^{2+}$-uptake blocking activity for nifedipine where none had been observed previously [8] suggests that FOURPHIT may displace endogenous inhibitors of the DHP binding site [13]. This effect of FOURPHIT may also account for the increases in [^{3}H]nitrendipine binding following the treatment of neuro-

Table 1. Effects of FOURPHIT and METAPHIT treatment and PCP and nifedipine on $^{45}Ca^{2+}$ uptake into mouse brain neurons

Treatment*	$^{45}Ca^{2+}$ uptake (nmol/mg protein)		
	Stimulated†	Resting†	Net†
Buffer† (control)	3.4 ± 0.2 (23)	1.2 ± 0.2 (23)	2.3
FOURPHIT (10 μM)	3.9 ± 0.2 (26)	1.2 ± 0.1 (15)	2.7
FOURPHIT (100 μM)	2.9 ± 0.1‡ (26)	1.2 ± 0.1 (15)	1.7
METAPHIT (10 μM)	3.5 ± 0.2 (26)	1.4 ± 0.2 (14)	2.0
METAPHIT (100 μM)	2.7 ± 0.2‡ (26)	1.2 ± 0.2 (15)	1.4
PCP (100 μM)	3.4 ± 0.2 (10)	0.9 ± 0.1 (10)	2.5
Nifedipine (10 μM)	3.5 ± 0.3 (6)	1.1 ± 0.2 (6)	2.4
Nifedipine (10 μM) + FOURPHIT (10 μM)	2.7 ± 0.2‡ (12)	0.9 ± 0.3 (12)	1.8
Nifedipine (10 μM) + METAPHIT (10 μM)	3.2 ± 0.1 (12)	1.2 ± 0.3 (12)	2.0

All values are the means ± SE of the number of experiments shown in parentheses.

* After initially sieving the mouse brains through Nitex screen, the unwashed neurons were incubated in a physiologic solution (see Methods) with either FOURPHIT, METAPHIT or PCP at the concentrations indicated for 15 min. The neurons were then processed as described by Skattebol and Triggle [8], resulting in a washing or removal of unbound drug. All drugs were prepared in distilled water and diluted in the same.

† For control, an aliquot of distilled water equivalent to that for delivering drugs was added to the neuron preparation. The conditions for stimulated, resting, and net $^{45}Ca^{2+}$ uptake are those described for the noncholine containing buffer condition (see Ref. 8). $^{45}Ca^{2+}$ uptake was measured at 5 sec.

‡ Significantly different from control, $P < 0.05$ (unpaired Student's *t*-test).

nal membranes with FOURPHIT. Alternatively, although highly speculatory, FOURPHIT may acylate membrane pumps involved in controlling the translocation of membrane calcium, thereby facilitating the observation of an inhibitory effect for nifedpine on voltage-dependent calcium channel function.

This study has revealed that PCP may inhibit neuronal calcium channels by membrane destabilization and a direct interaction(s) with components of the calcium channel proper. The inhibition of voltage-dependent sodium, potassium and calcium channels by PCP may provide a plausible explanation for its neurotransmitter release stimulating and blocking effects.

In summary, the effects of phencyclidine (PCP) and the acylating derivatives FOURPHIT and METAPHIT were investigated on K^+-stimulated $^{45}Ca^{2+}$ uptake into a preparation of mouse brain neurons. PCP produced a concentration-dependent inhibition of depolarization-dependent $^{45}Ca^{2+}$ uptake (IC_{50} $4.0 \pm 0.2\ \mu M$) which was reversed by washing of the neurons. Treatment of neurons with a $100\ \mu M$, but not a $10\ \mu M$ concentration of the PCP acylating drugs FOURPHIT and METAPHIT followed by washing resulted in a significant inhibition (25–40%) of K^+-stimulated $^{45}Ca^{2+}$ uptake. Nifedipine ($10\ \mu M$) produced a significant inhibition (33%) of $^{45}Ca^{2+}$ uptake only in neurons treated with $10\ \mu M$ FOURPHIT. These results suggest that PCP can interact with neuronal calcium channels in several ways to alter their function.

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