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# SHORT COMMUNICATIONS

## Displacement of [$^3$H]phencyclidine binding to *Torpedo* electric organ membrane by calcium channel antagonists

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Recently, Quirion and Pert [1] reported that certain calcium antagonists displace [$^3$H]phencyclidine ([$^3$H]PCP) binding in rat brain. They found that verapamil, and its methoxy derivative, D-600, were potent displacers of [$^3$H]PCP binding with $K_i$ values of 1.4 and 1.7 $\mu$M respectively. In contrast, two calcium antagonists of the 1,4-dihydropyridine series, nifedipine and nitrendipine, were very weak displacers of [$^3$H]PCP binding, with $K_i$ values $>100$ $\mu$M. Eldefrawi *et al.* [2] reported the presence of a high-affinity binding site for [$^3$H]PCP in crayfish abdominal muscle ($K_D = 13.5$ nM), and this [$^3$H]PCP binding was also displaced by calcium antagonists. The $IC_{50}$ values for displacement of [$^3$H]PCP binding to crayfish muscle by these antagonists, as determined by extrapolation from the figure presented by Eldefrawi *et al.* [2], were: verapamil $\simeq 0.06$ $\mu$M, (+)-D-600 $\simeq$ 0.2 $\mu$M, (−)-D-600 $\simeq 0.4$ $\mu$M, and nifedipine $\simeq 10$ $\mu$M. Hence, in crayfish muscle, as in rat brain, nifedipine was considerably weaker than verapamil in displacement of [$^3$H]PCP binding, although in crayfish muscle all of the antagonists were more potent than in rat brain.

In our laboratory, we have been studying the mechanism of action of calcium channel antagonists [3]. As part of our studies, we examined the influence of calcium antagonists on [$^3$H]PCP binding to microsacs isolated from the electric organ of *Torpedo californica*, which have been shown to be a rich source for the specific binding of [$^3$H]PCP [4]. Consistent with the findings in rat brain and crayfish muscle, we find that verapamil potently displaced [$^3$H]PCP binding to *Torpedo* microsacs. However, in contrast to these other tissues, in *Torpedo* microsacs, some calcium channel antagonist of the 1,4-dihydropyridine series, as well as the diphenylmethylalkylamine series, were more potent than verapamil as antagonists of [$^3$H]PCP binding. $CaCl_2$ also displaced [$^3$H]PCP binding to *Torpedo* microsacs. These studies suggest that the calcium channel antagonists may interact with the ionic channel associated with the nicotinic acetylcholine receptor in *Torpedo* electric organ membranes. A preliminary report of these findings has been published previously [5].

### *Materials and methods*

The methods for the preparation of microsacs from *T. californica* and the binding of [$^3$H]PCP were adapted from the procedures of Eldefrawi *et al.* [4], as described previously [6] with minor modifications. Binding was determined in a total volume of 1 ml by incubation of 250 $\mu$g of microsac protein with 2 pmoles (0.1 $\mu$Ci) of [$^3$H]PCP, 10 nmoles of carbachol, and graded amounts of test agents in 50 mM Tris–HCl, pH 7.4. Agents tested for their effect on [$^3$H]PCP binding were preincubated with microsac protein for 6 min at room temperature (20°). Binding was then initiated by the simultaneous addition of [$^3$H]PCP and carbachol, and incubation was continued for an additional 6 min (20°). Incubations were terminated by filtration through Whatman GF/B glass fiber filters, and the filters were washed three times with 3 ml of 50 mM Tris–HCl pH 7.4. The amount of [$^3$H]PCP bound to microsacs was determined by counting the filters in 4 ml of Liquiscint (National Diagnostics). Filter blanks were routinely counted and never exceeded 1.5% of the total counts of [$^3$H]PCP added to the assay. We have found that carbachol enhances the binding of [$^3$H]PCP 15-fold, and the effect is maximal at 10 $\mu$M carbachol. A similar enhancement of [$^3$H]PCP binding was reported for acetylcholine [4]. [$^3$H]PCP (48 Ci/mmole) was obtained from New England Nuclear. Calcium channel antagonists were gifts from the following companies: verapamil, Knoll Pharmaceuticals; nicardipine, Syntex Laboratories; nifedipine, Pfizer Pharmaceuticals; and nimodipine, Miles Laboratories. Stock solutions (1 mM) of calcium antagonists were prepared: verapamil and nicardipine in water, and the other antagonists in 100% ethanol. Dilutions of the stock solutions were then made in water. Controls for the appropriate amount of ethanol were run in all cases.

[$^{125}$I]-α-Bungarotoxin ([$^{125}$I-α-BTX) binding to *Torpedo* microsacs was assayed as described previously [6].

### *Results*

Under the conditions of this assay (2 nM [$^3$H]PCP and 10 $\mu$M carbachol), an average of $140 \pm 10$ fmoles ($5846 \pm 291$ cpm) (S.E., N = 34) of [$^3$H]PCP bound to 250 $\mu$g of microsac protein. Since this binding is reduced to filter blank levels upon boiling of the microsacs, or addition of high concentrations of local anesthetics, this binding is considered specific [4, 6]. As shown in Fig. 1, the binding of [$^3$H]PCP to these microsacs was displaced by $CaCl_2$ and several structurally unrelated classes of calcium channel antagonists, including verapamil, the diphenylmethylalkylamines—cinnarizine and flunarizine, and the 1,4-dihydropyridines—nicardipine, nimodipine and nifedipine. The concentrations of these drugs that inhibit 50% of the binding of [$^3$H]PCP to microsacs ($IC_{50}$), as determined from the curves in Fig. 1, are: verapamil, 4.8 $\mu$M; flunarizine, 2.7 $\mu$M; cinnarizine, 1.5 $\mu$M; nicardipine, 1.4 $\mu$M; nimodipine, 2.5 $\mu$M; and nifedipine, 9.8 $\mu$M. In addition to the calcium channel antagonists, $CaCl_2$ itself also displaced [$^3$H]PCP binding, although at higher concentrations. The $IC_{50}$ for displacement of [$^3$H]PCP binding by $CaCl_2$ was 2.3 mM (Fig. 1, top panel).

The mechanism of inhibition of [$^3$H]PCP binding by verapamil and nicardipine was further examined by Dixon plot analysis [7], as shown in Fig. 2. By this analysis, both verapamil and nicardipine competitively inhibited the binding of [$^3$H]PCP with apparent $K_i$ values of 1.5 and 0.5 $\mu$M respectively.

These observations indicate that the potency of verapamil for displacement of [$^3$H]PCP binding is very similar in both *Torpedo* electric organ and rat brain membranes. Displacement by 1,4-dihydropyridines, however, is quite different in these tissues. In contrast to rat brain and crayfish muscle, where 1,4-dihydropyridines were more than two orders of magnitude less potent than verapamil, in *Torpedo* membranes, the 1,4-dihydropyridines, nimodipine and nicardipine, were 2.0- and 3.4-fold more potent than verapamil, respectively, and the 1,4-dihydropyridine nifedipine was only 2-fold less potent than verapamil. Moreover, two calcium antagonists of the diphenyl-

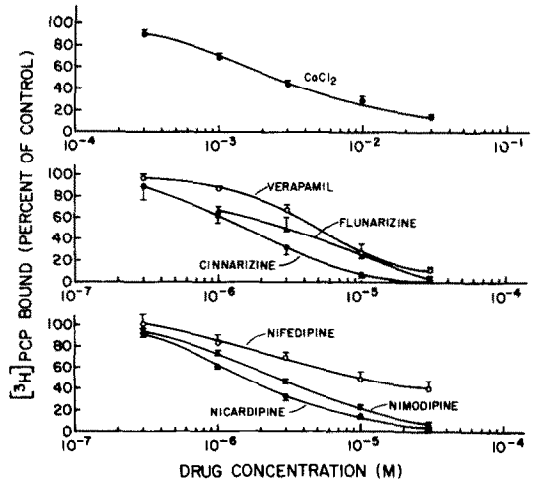


Fig. 1. Displacement of [$^3$H]PCP binding from *T. californica* electric organ membrane by calcium and calcium antagonists. [$^3$H]PCP binding to *T. californica* microsacs was determined as described in the text following a 6-min preincubation with different concentrations of antagonists, as indicated. Results are presented as percent of [$^3$H]PCP bound relative to controls containing no inhibitors. The results represent the mean ± S.E. of four to eight separate determinations for each antagonist.

methylalkylamine class, flunarizine and cinnarizine, were also potent displacers of [$^3$H]PCP binding to *Torpedo* microsacs; they were each 1.8- and 3.2-fold more potent than verapamil respectively.

Evidence suggests that, in *Torpedo* electric organ membranes, [$^3$H]PCP binds primarily to the ionic channel associated with the nicotinic acetylcholine receptor [4]. Since nicotinic agonists markedly enhance [$^3$H]PCP binding in these membranes, the calcium antagonists could cause inhibition of [$^3$H]PCP binding through an indirect effect on the binding of carbachol to the nicotinic receptor. For this reason, we examined the effects of the calcium antagonists on the binding of [$^{125}$I]-$\alpha$-BTX, a specific antagonist of the nicotinic acetylcholine receptor, to these *Torpedo* microsacs. At the concentrations at which they produce 50% inhibition of [$^3$H]PCP binding, none of the calcium antagonists used in this study inhibit [$^{125}$I]-$\alpha$-BTX binding, suggesting that they do not interact with the $\alpha$-BTX binding site.

*Discussion*

The observation that both calcium and calcium channel antagonists displaced [$^3$H]PCP binding to *Torpedo* electric organ membranes supports the suggestion of Quirion and Pert [1] that the site of binding of [$^3$H]PCP might be closely associated with a calcium channel. Based on the potency differences seen for the 1,4-dihydropyridines, however, the calcium channel antagonist and PCP binding sites in *Torpedo* appear to differ from those in rat brain and crayfish muscle. In rat brain and crayfish muscle, the 1,4-dihydropyridines were about two orders of magnitude less potent for displacement of [$^3$H]PCP binding than verapamil. In contrast, in *Torpedo* microsacs, with the exception of nifedipine, the 1,4-dihydropyridines were several-fold more potent than verapamil in displacement of [$^3$H]PCP binding.

Little is know about the actual structure of the binding site(s) for [$^3$H]PCP in rat brain and crayfish muscle. In contrast, in *Torpedo* membranes, [$^3$H]PCP binds predominantly to the subunits of the nicotinic acetylcholine receptor-ionic channel complex, as determined by photoaffinity labeling; and, moreover, carbachol specifically enhances the binding of [$^3$H]PCP and [$^3$H]azido-PCP to either the alpha, beta, or delta subunits of the complex, depending upon the species of *Torpedo* examined [9, 10]. The observation that nicotinic agonists enhanced the binding of [$^3$H]PCP (this study and Refs. 4 and 11) suggests that [$^3$H]PCP may bind preferentially to the agonist activated state of the channel associated with the acetylcholine receptor.

Although the ionic channel associated with the acetylcholine receptor conducts mainly $Na^+$ and $K^+$ ions [12], it is clearly not ion specific and has been shown to conduct $Ca^{2+}$ at 22% of the rate at which it conducts $Na^+$ [12, 13]. Our observation that both calcium and a variety of calcium antagonists displaced agonist-activated [$^3$H]PCP binding is consistent with the concept that the acetylcholine receptor associated ionic channel can function as a calcium channel

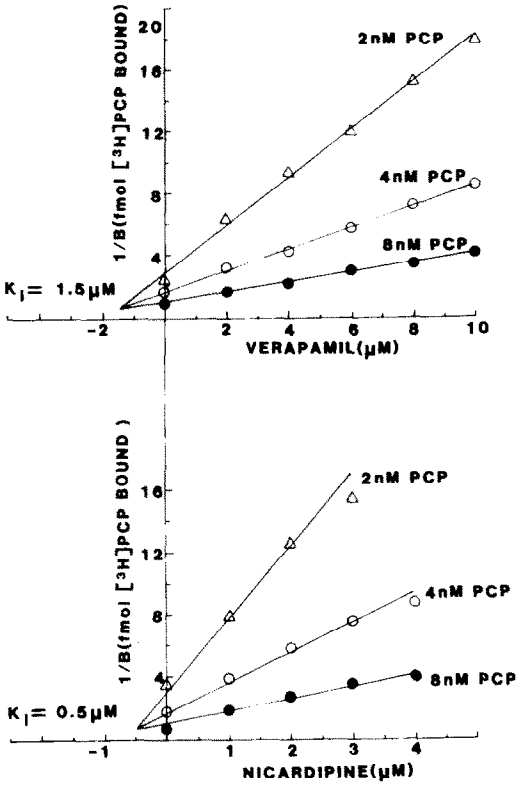


Fig. 2. Dixon plot analysis of the inhibition of [$^3$H]PCP binding to *T. californica* electric organ membrane by calcium antagonists. [$^3$H]PCP binding to *T. californica* microsacs was determined as described in the text, with the exception that 2, 4, and 8 nM [$^3$H]PCP concentrations were employed. Various concentrations of verapamil (top panel) or nicardipine (bottom panel) were tested for inhibition of [$^3$H]PCP binding by a 6-min preincubation with microsacs prior to the addition of [$^3$H]PCP. Results are plotted according to Dixon [7]. Since the lines on these Dixon plots intersect above, and not on, the abscissa, it rules out noncompetitive inhibition. This type of plot does not, however, in itself distinguish between competitive and linear mixed-type inhibition. However, when the slope of the lines in the Dixon plots are plotted vs 1/[PCP], linear lines are obtained for both verapamil and nicardipine which transect the origin (not shown); this clearly rules out linear mixed-type inhibition, and indicates that inhibition by both of these agents is competitive [8].

and that [$^3$H]PCP may be binding to this channel. It is noteworthy that these calcium antagonists also block $\alpha_1$-adrenergic and muscarinic acetylcholine receptors in the same concentration range in which they inhibit [$^3$H]PCP binding [14, 15], particularly since occupation of $\alpha$-adrenergic and muscarinic receptors is frequently coupled to changes in calcium permeability [16, 17]. Although little is known structurally about other channels permeable to calcium, the acetylcholine receptor-ionic channel complex from *Torpedo* membrane has been purified to homogeneity and has been well characterized [18]. Hence, the ionic channel associated with the acetylcholine receptor may provide a useful system for analysis of the precise mechanism by which calcium channel antagonists interact with a receptor–channel complex.

In sum, we have shown that the specific binding of [$^3$H]PCP to electric organ membrane isolated from *T. californica* was displaced by $CaCl_2$ and several structurally different classes of calcium channel antagonists, including verapamil, the diphyenlmethylalkylamines, and the 1,4-dihydropyridines. The calcium antagonists appeared to inhibit [$^3$H]PCP binding competitively. The $IC_{50}$ values for displacement of [$^3$H]PCP binding by these compounds were: $CaCl_2$, 2.3 mM; verapamil, 4.8 $\mu$M; flunarizine, 2.7 $\mu$M; cinnarizine, 1.5 $\mu$M; nicardipine, 1.4 $\mu$M; nimodipine, 2.5 $\mu$M; and nifedipine, 9.8 $\mu$M. These studies suggest that calcium channel antagonists may interact with the ion channel associated with the nicotinic acetylcholine receptor.

*Addendum*—Subsequent to the submission of this manuscript, a detailed analysis of the effects of calcium on [$^3$H]PCP binding to acetylcholine receptor enriched membrane from *Torpedo* electric organ appeared [19]. It was found that, in the presence of 0.2 mM carbachol, calcium decreases the binding of [$^3$H]PCP with an $IC_{50} \simeq 1$ mM. The inhibition of [$^3$H]PCP binding by calcium results from a decrease in the equilibrium affinity for PCP in the presence of carbachol.

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# Identification of 6-mercaptopurine riboside in patients receiving 6-mercaptopurine as a prolonged intravenous infusion

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6-Mercaptopurine (6-MP) is an analog of hypoxanthine that has been in clinical use for over 30 years [1]. Its value as a remission maintenance agent for the treatment of acute lymphoblastic leukemia has been well established [2]. To be biologically active, 6-MP must first undergo intracellular conversion to a nucleotide, thioinosinic acid (TIMP) [3]. This compound is then converted to thiguanine ribonucleotide and deoxyribonucleotide, and these compounds have been shown to subsequently be incorporated into RNA and DNA [4]. Following administration of 6-MP in man, the drug undergoes extensive metabolism initiated by the enzyme xanthine oxidase. The major product of this pathway is 6-thiouric acid (TU) with 6-thioxanthine (TX) being an intermediary metabolite [3, 5]. Methylation of 6-MP has also been shown to occur in man [5]. Following metabolism, 6-MP and its catabolites are eliminated in the