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m-Azido-Phencyclidine Covalently Labels the Rat Brain PCP Receptor, a Putative K Channel

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Phencyclidine (PCP) is a schizophrenomimetic drug of abuse. PCP binds with high affinity (apparent dissociation constant, $K_D < 10^{-6}$ M) to rat brain membranes and blocks, selectively, a voltage-gated, noninactivating K channel found in rat brain synaptosomes (presynaptic nerve terminals). Thus, it has been proposed that the high-affinity PCP receptor in brain is this K channel. Consistent with this hypothesis, we now show that several K channel blockers displace ^{3}H-PCP from the rat brain receptor. Additionally, we have used a photolabile analog of PCP, *m*-azido-PCP (Az-PCP), to identify the brain PCP receptor/putative K channel.

In the dark, Az-PCP bound reversibly to 2 classes of sites on rat brain synaptic membranes [$K_D = 0.14 \pm 0.01$ μM ($n = 5$) for high-affinity binding, and $K_D = 255 \pm 55$ μM for low-affinity binding]. Competitive binding studies between Az-^{3}H-PCP and nonlabeled PCP analogs, and between Az-PCP and several tritiated PCP analogs, indicated that the high-affinity Az-PCP binding site is the high-affinity PCP receptor. Several aminopyridines (APs) and tetraalkylamines (TAAs), which are known to block K channels in excitable cells, were also found to displace ^{3}H-PCP from its high-affinity binding site on rat brain synaptic membranes. The rank order of potency for displacement of ^{3}H-PCP from this site for the APs was 4-AP ≃ 3,4-diAP > 2-AP ≫ 3-AP; for the TAAs it was TBA > TEA ≫ TMA (the tetra-butyl, ethyl, and methyl amines, respectively). These sequences parallel those observed for block of some K channels and/or enhancement of neurotransmitter release.

Az-^{3}H-PCP was covalently attached to rat brain membranes by photoactivation; subsequent separation of the membrane polypeptides by SDS-PAGE, followed by fluorography, revealed that several polypeptides incorporated the label. Labeling of 2 polypeptides, with apparent molecular weights of 95,000 and 80,000, was blocked by PCP and by its behaviorally active thienyl analog, TCP, with little reduction of the label incorporated onto any other polypeptide. 4-AP and TBA also specifically blocked the covalent attachment of Az-^{3}H-PCP to the 95 and 80 kDa polypeptides. We therefore conclude that the high-affinity PCP receptor in rat brain consists of 95 and 80 kDa polypeptides and that these 2 polypeptides are also components of a voltage-gated K channel.

Phencyclidine (1-[1-phenylcyclohexyl] piperidine; PCP) is a dissociative anesthetic and drug of abuse that in man produces a toxic psychosis with many schizophrenia-like symptoms (Domino and Luby, 1981). A high-affinity (apparent $K_D < 10^{-6}$M) binding site for PCP has been described in rat brain (Blaustein and Ickowicz, 1983; Hampton et al., 1982; Mendelsohn et al., 1984; Vincent et al., 1983; Zukin et al., 1983). This receptor appears to be responsible for the abnormal behavior induced by PCP because the affinities of PCP and its analogs for binding to the brain receptor correlate with their behavioral potencies (Lazdunski et al., 1983; Mendelsohn et al., 1984; Zukin and Zukin, 1979).

PCP and its analogs have been shown to block selectively a voltage-gated, noninactivating K channel that is present in isolated rat brain presynaptic nerve terminals (synaptosomes) (Bartschat and Blaustein, 1986a). Selective block of this channel by "sigma" opiates with PCP-like activity has also been observed (Bartschat and Blaustein, 1986b). The potencies of PCP and its analogs for block of this K channel correlate with the affinities of these compounds for binding to the high-affinity receptor (Bartschat and Blaustein, 1986a). Likewise, the stereospecificities of "sigma" opiates for inhibition of high-affinity ^{3}H-PCP binding (Bartschat et al., 1985; Mendelsohn et al., 1984) and for block of the voltage-gated, noninactivating K channel (Bartschat and Blaustein, 1986b) are similar. Thus, it was proposed that the high-affinity PCP receptor in the brain may be this K channel (Bartschat and Blaustein, 1986a; Blaustein and Ickowicz, 1983).

In an effort to identify the mammalian brain PCP receptor, we employed an approach similar to that already used successfully to label the PCP receptor in *Torpedo* membranes (Haring et al., 1984; Heidmann et al., 1985). We synthesized a photolabile analog of PCP, Az-PCP, in both nonradiolabeled and tritiated (Az-^{3}H-PCP) forms. Our data indicate that this agent binds with high affinity to the PCP receptor of rat forebrain. Photolabeling with Az-^{3}H-PCP results in the specific incorporation of label onto 2 polypeptides; covalent labeling is blocked by PCP and by its thienyl analog TCP. The idea that the brain PCP receptor is a K channel is supported by our observation that 2 known K channel blockers, 4-aminopyridine (4-AP) and tetrabutylammonium (TBA), also block ^{3}H-PCP binding to the high-affinity sites on synaptic membranes and block the covalent attachment of Az-^{3}H-PCP onto the same 2 polypeptides, selectively. Some of these findings have been presented in preliminary form (Sorensen and Blaustein, 1985a, b).

Materials and Methods

Materials

^{3}H-PCP (49.9 Ci/mmol) was obtained from New England Nuclear (Boston, MA); ^{3}H-1-(1-thienylcyclohexyl) piperidine (^{3}H-TCP; 64 Ci/mmol) was obtained from New England Nuclear or from Research Products International (Mt. Prospect, IL). Amino-^{3}H-PCP (18 Ci/mmol) was custom tritiated by Amersham (Arlington Heights, IL). Unlabeled PCP, TCP, and amino-PCP were obtained from the National Institute on Drug Abuse (NIDA). HEPES, Tris, AP analogs, and tetraalkylamine (TAA) analogs were obtained from Sigma Chemical Co. (St. Louis, MO). 4-AP was also obtained from Aldrich Chemical Co. (Milwaukee, WI).

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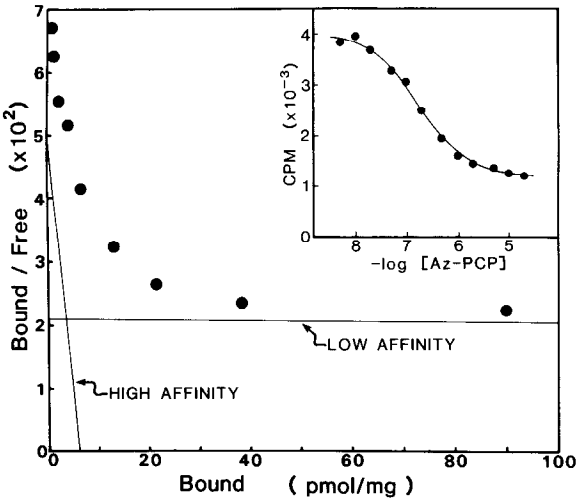


Figure 1. Binding of Az-^{3}H-PCP to rat brain synaptic membranes. The graph is the Scatchard plot calculated from data from a representative experiment in which the binding parameters were determined from the displacement of Az-^{3}H-PCP by unlabeled Az-PCP. To illustrate the high-affinity binding clearly, the 2 data points obtained with the highest concentrations of unlabeled Az-PCP (points at 1 and 2 × 10^{-5} M; see *inset*), which fall on the "low affinity" line, have been omitted. Plotted values were calculated from the displacement curve data (means of triplicate determinations) shown in the inset. The values were fitted to a 2-site model by computer-assisted curve-fitting analysis (Munson and Rodbard, 1980); the 2 calculated curves for the binding sites (high and low affinity) are indicated. The calculated binding parameters for this experiment were: high affinity, K_D = 0.147 μM, B_{max} = 6.0 pmol ligand/mg protein; low affinity, K_D = 225 μM, B_{max} = 3863 pmol ligand/mg protein.

Preparation of Az-PCP and Az-^{3}H-PCP

Az-PCP was prepared by diazotization of *m*-amino-PCP. All reaction mixtures were kept on ice. *m*-Amino-PCP (10 mg, 0.039 mmol) was dissolved in 10 ml of 3 N acetic acid. $NaNO_2$ (3.2 mg in 0.5 ml, 1.2 equiv.) was added, followed, after 15 min, by NaN_3 (3.3 mg in 0.5 ml, 1.3 equiv.). After an additional 15 min, the mixture was neutralized with 4 ml concentrated NaOH and then extracted with $CHCl_3$ (3 × 5 ml); the $CHCl_3$ extract was dried over $MgSO_4$ and evaporated to dryness under N_2. Az-PCP was stored in absolute ethanol. Successful incorporation of the azido moiety was determined from the UV absorbance spectrum of the compound. The absorbance spectrum of Az-PCP (maximum at 255 nm) changed upon photoactivation of the compound with UV light—this is characteristic of azido compounds (DeTraglia et al., 1978)—whereas the absorbance spectrum of amino-PCP (maximum at 250 nm) was unchanged after UV irradiation. The compound ran as a single spot during thin-layer chromatography on silica gel [solvent systems: methanol/NH_4OH (99:1), R_f = 0.68; and *n*-butanol/acetic acid/H_2O (25:8:10), R_f = 0.47].

A similar but scaled-down procedure was used to prepare Az-^{3}H-PCP. Amino-^{3}H-PCP (1 mCi; 0.056 μmol) was dried under N_2 and dissolved in 20 μl of 3 N acetic acid. $NaNO_2$ (6.5 μg in 1 μl) and NaN_3 (6.6 μg in 1 μl) were added as described above. The mixture was neutralized with 8 μl concentrated NH_4OH, extracted with $CHCl_3$ (3 × 100 μl), and dried over $MgSO_4$. The Az-^{3}H-PCP was evaporated to dryness under N_2 and stored in absolute ethanol. Az-^{3}H-PCP comigrated with Az-PCP on thin layer chromatography.

Ligand binding assay

Ligand binding was measured by determining the concentration of unlabeled ligand required to displace a tritiated ligand. Synaptic membranes were prepared from rat forebrain by the method of Salvaterra and Matthews (1980). The membranes were incubated at 37°C in 20 mM Tris/HEPES, pH 7.0, containing a constant amount of tritiated ligand and increasing amounts of the unlabeled compounds (as indicated in the tables and figure legends) in a total volume of 0.5 ml. When Az-PCP or Az-^{3}H-PCP was present in the binding mixture, the assay was performed in the dark to avoid photolysis. Binding reactions were initiated by the addition of membranes (usually 600 μg protein). After 30 min to allow the mixtures to reach equilibrium, 4 ml of cold wash buffer (20 mM Tris/HEPES, pH 7.0) was added to the tubes, and the contents were rapidly applied to Whatman GF/B filters presoaked in 0.1% polyethyleneimine to reduce nonspecific binding (Hampton et al., 1982). The filters and retained membranes were washed twice with 4 ml of the same wash buffer and counted for ^{3}H by liquid scintillation spectroscopy. Each assay was carried out in triplicate. Appropriate blank, protein-free values for each data point were subtracted from the counts obtained in the presence of protein.

Table 1. Binding of PCP analogs to the rat brain high-affinity PCP receptor

Test compound	^{3}H-labeled ligand	Binding parameters for displacement of ^{3}H-labeled ligand: $K_{0.5}$ (μM)	B_{max} (pmol/mg protein)
TCP	^{3}H-TCP	0.023 ± 0.006	2.3 ± 0.2 (3)[a]
	Az-^{3}H-PCP	0.031	5.0
m-amino-PCP	*m*-amino-^{3}H-PCP	0.043	2.9
	Az-^{3}H-PCP	0.055	4.3
PCP	^{3}H-PCP	0.065 ± 0.005	2.8 ± 0.3 (5)[a]
	Az-^{3}H-PCP	0.088 ± 0.005	5.9 ± 0.7 (3)[a]
Az-PCP	Az-^{3}H-PCP	0.139 ± 0.011	5.9 ± 0.6 (5)[a]
	^{3}H-PCP	0.180 ± 0.012	2.3 ± 0.4 (4)[a]
	^{3}H-TCP	0.148	2.3
	m-amino-^{3}H-PCP	0.183	1.8

The ability of unlabeled (test) compounds to displace tritium-labeled ligands was determined as described in Materials and Methods. The binding mixtures contained 20 mM Tris/HEPES, pH 7.0, increasing amounts of the test compound, and either 5 nM ^{3}H-PCP, 5 nM Az-^{3}H-PCP, 5 nM *m*-amino-^{3}H-PCP, or 2.5 nM ^{3}H-TCP, as indicated. The $K_{0.5}$ values (μM) are the apparent half-maximal binding constants for the displacing (unlabeled) compound; B_{max} values (pmol ligand bound/mg membrane protein) indicate the estimated number of high-affinity binding sites for the labeled ligand.

[a] Data are means ± SEM of 3–5 experiments as indicated in parentheses.

Photolabeling procedure

To identify the individual membrane polypeptides to which Az-^{3}H-PCP covalently attached, synaptic membranes were incubated in the dark at 0°C in either 5 mM Tris/HEPES, pH 7.0, or 10 mM sodium phosphate, pH 7.0, containing 1.0 μM Az-^{3}H-PCP and various test compounds. After 60 min, the suspensions were filtered and washed as described above for the ligand binding assay. The washed filters were irradiated for 5 min under shortwave (254 nm) or for 15 min under long-wave (366 nm) UV light with a fixed 15 W UV lamp source (Ultra-Violet Products, Inc., San Gabriel, CA) at a distance of 6–8 cm to promote covalent attachment of the ligand. (Essentially identical results were obtained with irradiation at the 2 wavelengths.) The filters were incubated overnight in SDS–dissociation buffer (5 mM Tris, pH 8.0, 3% SDS, 5% β-mercaptoethanol and 10% glycerol). Finally, the solubilized membrane proteins were separated by SDS-PAGE (DeBlas et al., 1979). The fixed, unstained gels were impregnated with "Fluoro-Hance" (Research Products International), dried, and exposed to Kodak X-Omat AR film to obtain fluorographic patterns of the labeled polypeptides.

Protein was determined by the method of Markwell et al. (1978). Binding constants were determined through computer-assisted curve-fitting analysis (LIGAND program) for a 2-site model (Munson and Rodbard, 1980).

Results and Discussion

Az-PCP binding to the rat brain PCP receptor

The binding of Az-PCP and other PCP analogs to brain synaptic membranes was determined from the displacement of a tritium-

labeled analog by an unlabeled analog. Data from an experiment in which unlabeled Az-PCP was used to displace Az-^{3}H-PCP are shown in Figure 1. Previously, we reported that PCP binds to 2 classes of sites in rat brain membranes (Blaustein and Ickowicz, 1983; Sorensen and Blaustein, 1985a). Az-PCP also binds to 2 classes of sites (Fig. 1): high-affinity sites with $K_D = 0.139 \pm 0.010$ μM and $B_{max} = 5.9 \pm 0.5$ pmol ligand/mg protein, and low-affinity sites with $K_D = 255 \pm 55$ μM and $B_{max} = 4849 \pm 1113$ pmol ligand/mg protein (means ± SE of 5 experiments). The low-affinity binding probably results from interactions between the highly hydrophobic PCP and Az-PCP molecules and the hydrophobic regions of many integral membrane proteins and lipids (Heidmann et al., 1985).

As shown in Table 1, similar values were obtained for the apparent affinity of Az-PCP for high-affinity binding to synaptic membranes irrespective of which tritiated PCP analog was used in the displacement assay. Likewise, the apparent affinities of the other PCP analogs (PCP, TCP and *m*-amino-PCP) for these sites were similar, whether the tritiated form of the respective ligand or Az-^{3}H-PCP was employed in the assay. These data indicate that Az-PCP binds with high affinity to the same brain receptor sites as those to which PCP, TCP, and *m*-amino-PCP bind.

Evidence that Az-PCP binds covalently to the high-affinity PCP receptor was obtained by showing that it inhibits subsequent ligand binding. Synaptic membranes were equilibrated in the dark with excess (2 μM) unlabeled Az-PCP. The suspensions were then either kept in the dark or irradiated with UV to photolyze the Az-PCP. The membranes were subsequently washed to remove excess Az-PCP and were assayed for the ability to bind ^{3}H-PCP or ^{3}H-TCP to determine the availability of high-affinity binding sites. Irradiation in the absence of Az-PCP reduced the available number of high-affinity sites (B_{max}) by 25%; irradiation in the presence of Az-PCP produced a further reduction of 14 + 2% ($n = 3$), with negligible effect on the K_D. This suggests that the efficiency of covalent attachment of Az-PCP to the high-affinity sites is about 14%, which seems reasonable for an aryl-substituted nitrene (Cavalla and Neff, 1985).

Covalent labeling of the membrane polypeptides

The preceding results indicate that Az-PCP binds covalently, and with high affinity, to the rat brain PCP receptor. Therefore, Az-^{3}H-PCP was used to identify this receptor. Synaptic membranes were equilibrated with 1 μM Az-^{3}H-PCP at 0°C, with or without a 100- to 1000-fold excess of unlabeled PCP or TCP. [Our unpublished observations indicate that the binding of PCP and its analogs to the PCP receptor is temperature sensitive: The concentration of Az-^{3}H-PCP used (1 μM) is close to the K_D for the high-affinity site (0.95 μM at 0°C), but <1% of the K_D for the low-affinity site (500 μM at 0°C).] The radioactive ligand was then covalently attached to the membranes by photoactivation with UV light. Subsequent analysis of the solubilized membranes by SDS-PAGE and fluorography showed incorporation of radioactivity into several polypeptides (Fig. 2); non-irradiated samples did not incorporate the label (not shown). The presence of excess unlabeled PCP (0.5 mM) in the incubation medium clearly reduced the covalent labeling of 2 polypeptides, with apparent molecular weights (M_r) of 80,000 and 95,000.

The dense, ~95 kDa polypeptide band observed with Coomassie blue staining (Fig. 2, lane CB) probably corresponds to the α subunit of the Na,K-ATPase (Mahler, 1977; Sweadner, 1979). Control experiments with red blood cell and kidney membranes (not shown) indicate that Az-^{3}H-PCP does not label the Na,K-ATPase. Also, using an assay for solubilized membrane proteins, we found that ^{3}H-PCP does not bind to purified dog kidney Na,K-ATPase (R. G. Sorensen and M. P. Blaustein, unpublished observations). Presumably, the labeled polypeptide is present in low concentration and migrates close to the α subunit of the Na,K-ATPase (Fig. 2).

The 80 kDa polypeptide was only weakly labeled in some experiments (e.g., see Fig. 2*A*). This might suggest that it is a proteolytic cleavage product of the larger polypeptide, but inclusion of several protease inhibitors (1 mM EDTA, 0.5 mM phenylmethyl sulfonyl fluoride, and 2.5 units/ml aprotinin) in all solutions did not prevent labeling of the 80 kDa polypeptide. Therefore, we favor the view that the 95 kDa polypeptide contains the primary PCP binding site. The variable labeling of the 80 kDa polypeptide may indicate that it lies close to the 95 kDa polypeptide in the native membranes and, thus, may be part of the complex that makes up the PCP receptor. This would be analogous to experiments on the nicotinic ACh receptor (AChR), in which Az-^{3}H-PCP labels most of the subunits, even though only 1 appears to contain the primary PCP binding site (Haring et al., 1983).

While the present study was in progress, Haring et al. (1986) reported that 2 polypeptides, with apparent M_r 90,000 and 33,000, were specifically labeled by Az-^{3}H-PCP and were components of the high-affinity PCP receptor in rat hippocampus. Three other polypeptides, with M_r of 62,000, 49,000, and 40,000, were also labeled by Az-^{3}H-PCP in their experiments; however, further pharmacological characterization led Haring et al. to propose that these 3 polypeptides represent a second PCP receptor domain distinct from the high-affinity receptor. It seems probable that their 90 kDa polypeptide and our 95 kDa polypeptide are the same. We also sometimes observed weak labeling of a smaller polypeptide (M_r = 30,000–35,000) that may have been specifically displaced by PCP (Fig. 2*B*), although the results are not clear. This may correspond to the 33 kDa polypeptide described by Haring et al. In these respects, the 2 studies appear complementary. Haring et al. (1986) did not observe a specifically labeled 80 kDa polypeptide, but part of the difference may be due to our use of rat forebrain instead of hippocampus or to variable labeling of this polypeptide (Fig. 2). The data are difficult to compare in detail, however, because Haring et al. did not publish fluorograms of their gel patterns.

PCP also binds to the nicotinic AChR (Haring and Kloog, 1984; Heidmann et al., 1985; Oswald et al., 1984) and blocks the ion channel associated with this receptor (Albuquerque et al., 1981, 1983). However, 2 observations show that the PCP receptor in brain is distinct from the nicotinic AChR. First, PCP binds to the nicotinic AChR from *Torpedo* electric organ membranes with a K_D of about 6 μM (Haring and Kloog, 1984; Heidmann et al., 1985; Oswald et al., 1984; R. G. Sorensen and M. P. Blaustein, unpublished observations). This is 20- to 100-fold greater than the K_D for PCP binding to the brain receptor under comparable conditions. Second, both ^{3}H-PCP and Az-^{3}H-PCP covalently attach to polypeptides of apparent M_r 40,000, 50,000, and 66,000, which are the α, β, and δ subunits, respectively, of the *Torpedo* AChR (Haring et al., 1984; Heidmann et al., 1985); we obtained similar labeling patterns with *Torpedo* electroplax membranes (unpublished observations). These polypeptides are different from those photolabeled by Az-^{3}H-PCP in brain (Fig. 2).

TCP has a higher affinity for the brain receptor than does PCP (Table 1; see also Sorensen and Blaustein, 1985a; Vignon et al., 1983). Moreover, TCP is more potent than PCP both in its behavioral activity (Shannon, 1981; Vaupel et al., 1984) and in its ability to block presynaptic K channels (Bartschat and Blaustein, 1986a). Consistent with these observations, TCP also appeared to be more potent than PCP in blocking the covalent attachment of Az-^{3}H-PCP to the 95 and 80 kDa polypeptides (Fig. 2*B*). Scans of these fluorograms (with a Joyce-Loebl microdensitometer) indicated that, in the presence of 1 μM Az-^{3}H-PCP, 100 μM TCP displaced about 32% of the label in both the 95 and 80 kDa polypeptides, while 100 μM PCP displaced only about 22% of the label. The respective displacement values for

Table 2. Displacement of ^{3}H-PCP from the rat brain high-affinity PCP receptor by various aminopyridines and tetraalkylamines

Test compound	$K_{0.5}$ for displacement of ^{3}H-PCP from high-affinity sites (mM)
Aminopyridines	
4-AP	2.6 ± 0.1 (2)
3,4-diAP	3.1 ± 0.1 (3)
2-AP	8.8 ± 1.6 (3)
3-AP	53.9 ± 3.2 (3)
Tetraalkylamines	
TBA	1.6 ± 0.1 (2)
TEA	6.7 ± 0.8 (3)
TMA	33.6 ± 3.0 (4)

The ability of several aminopyridines and tetraalkylamines to compete with 5 nM ^{3}H-PCP for binding to synaptic membranes was measured as described in Materials and Methods. The $K_{0.5}$ values (mM) are the apparent half-maximal binding constants for the displacing compound (AP or TAA). The data are the means ± SEM of 2–4 experiments as indicated in the parentheses.

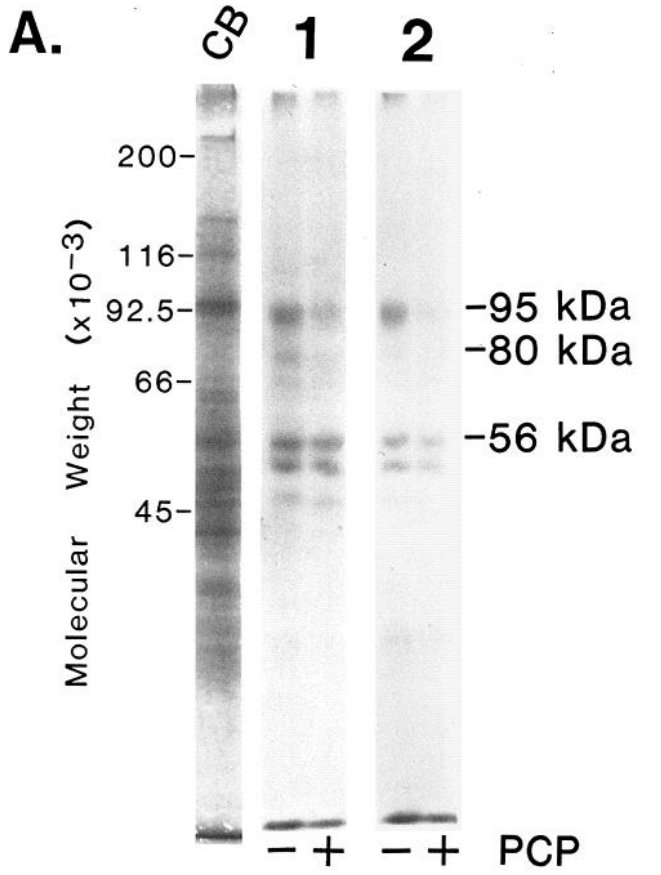
1000 μM TCP and PCP were about 68 and 59%. This is further evidence that these 2 polypeptides are part of the high-affinity PCP receptor complex.

Identification of the PCP receptor as a K channel

Studies in skeletal muscle (Tsai et al., 1980), cardiac muscle (Hadley and Hume, 1986), neuroblastoma (Tourneur et al., 1982), and presynaptic nerve terminals (Bartschat and Blaustein, 1986a) indicate that PCP blocks a voltage-gated K channel; this is manifested by an increase in action potential duration in the muscle and neuroblastoma preparations. In ^{86}Rb-loaded terminals, selective inhibition of a component of Rb efflux that corresponds to a voltage-gated, noninactivating K channel by PCP and its analogs closely parallels the behavioral potency of these agents (Bartschat and Blaustein, 1986a). Az-PCP also blocks this component of Rb efflux in nerve terminals (D. K. Bartschat and M. P. Blaustein, unpublished observations). These findings have led to the proposal that the PCP receptor in brain is a component of a K channel. This view is supported by the observation that the enantiomorphs of "sigma" opiate stereoisomer pairs with PCP-like behavioral effects also bind, stereospecifically, to the PCP receptor in brain membranes (Bartschat et al., 1985) and block the same presynaptic, voltage-gated K channel as does PCP (Bartschat and Blaustein, 1986b).

The data in Table 1 may provide some information about the K channel density in presynaptic nerve terminals, on the assumption that 1 PCP molecule is bound per channel. The density of PCP binding sites, about 2–3 pmol/mg synaptic membrane protein, can be compared to the density of Na channels (i.e., saxitoxin binding sites) in somewhat less "pure" rat brain synaptic membrane preparations: 1.4–4.7 pmol/mg protein (Angelides et al., 1985; Krueger et al., 1979). These data suggest that the ratio of Na channels to K channels in rat brain synaptic membranes may not be very different from the ratio of Na channels to delayed rectifier K channels, approximately 2–5:1, in squid giant axons and frog node of Ranvier (Hille, 1984).

Some APs and some TAAs are known to block voltage-gated K channels (Bartschat and Blaustein, 1985; French and Shoukimas, 1981; Stanfield, 1983; Thesleff, 1980; Thompson, 1977), including those in presynaptic nerve terminals (Bartschat and Blaustein, 1985; Stanfield, 1983; Thesleff, 1980). Therefore, we tested several APs and TAAs for their ability to displace ^{3}H-PCP from its high-affinity binding sites on synaptic membranes. The results (Table 2) show that millimolar concentrations of 4-AP, TBA, and tetraethylammonium (TEA) blocked ^{3}H-PCP binding to its brain receptor. Similar concentrations of these



compounds are required for block of the voltage-gated, noninactivating K channel in presynaptic terminals (Bartschat and Blaustein, 1985). The rank orders of potency for block of ^{3}H-PCP binding by the APs (4-AP ≃ 3,4-diAP > 2-AP ≫ 3-AP) and by the TAAs (TBA > TEA ≫ TMA, tetramethylammonium) parallel their ability to block K channels and/or enhance neurotransmitter release (Bartschat and Blaustein, 1986a; French and Shoukimas, 1981; Johns et al., 1976; Moritoki et al., 1978). These data are consistent with the idea that ^{3}H-PCP binds to a K channel.

On the basis of the foregoing observations, we examined the effects of 4-AP and TBA on the covalent labeling of synaptic membrane polypeptides by Az-^{3}H-PCP. As shown in Figure 2*B*, 5 mM 4-AP and 5 mM TBA both specifically blocked incorporation of label onto the 95 and 80 kDa polypeptides. The data in Table 2 and Figure 2*B* provide additional evidence that the rat brain PCP receptor is part of a voltage-gated, noninactivating K channel (Bartschat and Blaustein, 1986a). This is also supported by our observation that TBA and 4-AP are, respectively, a competitive inhibitor and an allosteric inhibitor of high-affinity PCP binding to synaptic membranes (R. G. Sorensen and M. P. Blaustein, unpublished observations). Therefore, the 95 and 80 kDa polypeptides that we labeled with Az-^{3}H-PCP appear to be subunits of both the PCP receptor and a K channel.

Intoxication with 4-AP (Spyker et al., 1980), as with PCP (Domino and Luby, 1981), causes dissociative behavior in man. These observations are consistent with the hypothesis (Albuquerque et al., 1981, 1983; Bartschat and Blaustein, 1986a) that the PCP receptor/K channel complex described in this report is involved in the behavioral disturbances generated by 4-AP and PCP. Our findings provide a means with which to initiate the purification and biochemical characterization of the PCP receptor/voltage-gated, noninactivating K channel from brain.

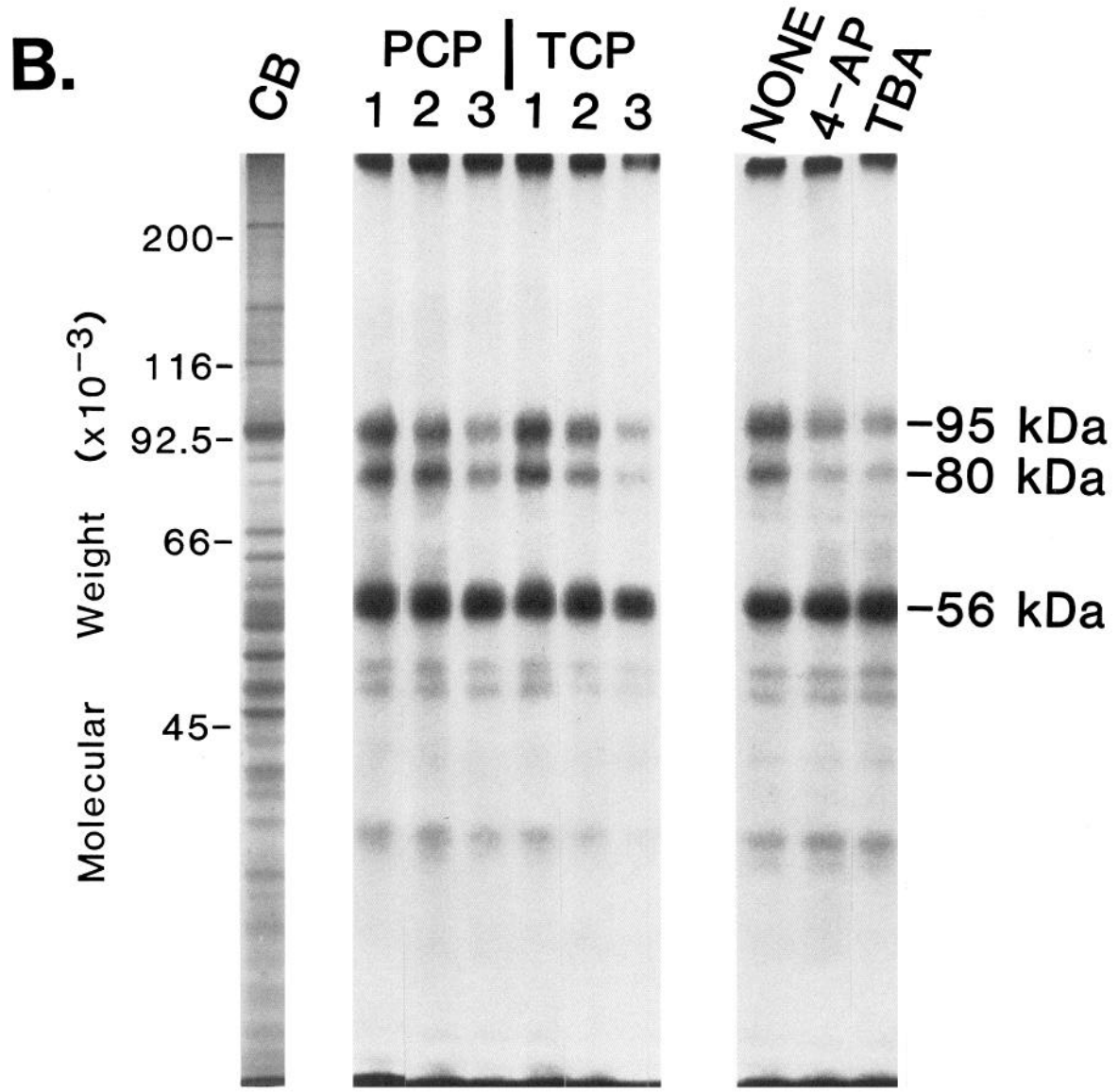


Figure 2. Fluorograms illustrating the covalent attachment of Az-^{3}H-PCP to brain membrane polypeptides. *A*, Synaptic membranes (0.63 mg/ml) were incubated in the dark at 0°C in either (*1*) 5 mM Tris/HEPES, pH 7.0, or (*2*) 5 mM sodium phosphate, pH 7.0, each containing 1.0 μM Az-^{3}H-PCP without (−) or with (+) 0.5 mM unlabeled PCP. Use of 5 mM Tris/HEPES buffer instead of 5 mM sodium phosphate did not significantly affect the level of ligand incorporation (compare *1* and *2*), whereas 50 mM Tris/HEPES buffer substantially reduced the level of incorporation (not shown). After 60 min, the binding mixtures were subjected to vacuum filtration to remove excess unbound ligand. Remaining procedures are described in Materials and Methods. Covalent attachment of the analog was promoted by irradiating the filters with longwave (366 nm) UV light for 15 min at a distance of 8 cm. The molecular weights of some labeled polypeptides (*numbers to the right*), as well as some molecular-weight standards (*numbers to the left*) are indicated (in kDa). *CB* indicates the polypeptide staining (Coomassie Brilliant blue) pattern of a gel run in parallel. The results show that excess PCP blocks the covalent labeling of the 80 and 95 kDa polypeptides but has little effect on the labeling of other membrane polypeptides such as the 56 kDa polypeptide. *B*, Synaptic membranes (2.1 mg/ml) were subjected to covalent labeling with 1 μM Az-^{3}H-PCP as in *A*, in 5 mM sodium phosphate, pH 7.0. Further additions to the binding mixtures were: for PCP and TCP, lanes 1–3 contained 1, 100, and 1000 μM unlabeled ligand, respectively; for 4-AP and TBA, 5 mM compound was added; *NONE* indicates that no unlabeled test substance was added. Photoactivation was induced by irradiation with shortwave (254 nm) UV light for 5 min. Differences in the migration rates of some of the labeled polypeptides between the fluorograms shown in *A* and *B* may have resulted from the use of different sources of SDS (*A*, from Sigma, St. Louis, MO; *B*, from Bio-Rad, Richmond, CA) in preparing the polyacrylamide gels.

Note added in proof: While this manuscript was in press, Petersen et al. (1986) reported that β-bungarotoxin (β-BuTX), a presynaptically acting polypeptide toxin from the venom of the snake *Bungarus multicinctus*, blocks a noninactivating K current in guinea pig dorsal root ganglion neurons. Rehm and Betz (1983) have shown that β-BuTX labels a 95 kDa polypeptide in chick brain membranes. These observations are consistent with the idea that the 95 kDa polypeptide from brain membranes labeled by Az-PCP (this report) and the one labeled by β-BuTX are identical and are, as we have suggested, part of a voltage-gated, noninactivating K channel in presynaptic nerve terminals.

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