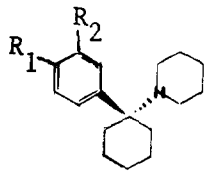

| CMPD.#: | 1(PCP) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $R_1$: | H | $NH_2$ | $NH_2$ | $NO_2$ | $NO_2$ | $NH_2$ |
| $R_2$: | H | H | F | Br | $18_F$ | $18_F$ |

## A POTENTIAL BIOASSAY FOR IDENTIFYING PCP AND SIGMA LIGANDS USING THE GUINEA-PIG VAS DEFERENS (GPVD). Vaupel, D. B. and T.-P. Su. NIDA-Addiction Research Center, Baltimore, MD 21224.

Electrically induced contractions of guinea-pig vas deferens were potentiated in a dose-responsive manner by sigma drugs such as d-pentazocine and d-SKF-10047 and also by phencyclidine-like drugs. Nonsigma drugs like morphine, buprenorphine, DADLE, U-50488 and l-ketocyclazocine did not potentiate contractions. The potentiations were antagonized by haloperidol and BW234U in an apparent noncompetitive fashion. Pretreatment with prazosin, an alpha$_1$-adrenoceptor antagonist, also appeared to antagonize noncompetitively the effects of d-pentazocine and phencyclidine. In contrast, prazosin antagonized the potentiation produced by methoxamine, a directly acting alpha$_1$-adrenoceptor agonist, in a competitive fashion in this tissue preparation. In a separate study, it was observed in this laboratory that maturing GPVD became less responsive to PCP. Therefore, a longitudinal study was conducted that evaluated the cumulative dose-response curves to PCP and d-SKF-10047. Field stimulated VD were tested from groups of GP weighing 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 kg. In 0.3–0.4 kg GP, PCP and d-SKF-10047 (0.3–300 $\mu$M) produced a peak potentiation of 250% of control amplitude and were equipotent. For the 0.5–0.7 kg groups, the peak d-SKF-10047 potentiation decreased slightly, but remained relatively stable at 200%, 205% and 195% respectively. In contrast, there were more pronounced decreases in the corresponding peak PCP potentiations, which were 127%, 115% and 140%. The peak effect of d-SKF-10047 decreased more prominently (165%) in the 0.8 kg group; the PCP potentiation was 110%. Thus the maturing GPVD appeared to lose its sensitivity to the stimualtory action of PCP while retaining most of its sensitivity to d-SKF-10047. Before characterizing this maturity related change in sensitivity to PCP, sigmaphin, a putative endogenous sigma ligand was bioassayed using a VD from a 0.45 kg GP. The sigmaphin was obtained from GP brain extracts through molecular sizing fractionation and ion-exchange chromatography. Sigmaphin potentiated the GPVD in a dose-responsive manner. Contractions in a single VD were potentiated to 240% of control by sigmaphin (1 mg/5 ml) and the peak effect of d-SKF-10047 was 360%. It is concluded that the GPVD may be a useful bioassay for separating the effects exerted by PCP and sigma drugs and that this tissue preparation may serve as an interesting tool for identifying putative endogenous ligands which interact with sigma and PCP receptors in the brain.

## INTERACTION OF MOLECULES IN THE PHENCYCLIDINE SERIES WITH THE DOPAMINE UPTAKE SYSTEM. CORRELATION WITH THEIR BINDING PROPERTIES ON THE PHENCYCLIDINE RECEPTOR. BINDING PROPERTIES OF [$^3$H]GK13, A NEW PCP ANALOG, TO THE DOPAMINE UPTAKE SYSTEM. Vignon, J., C. Cerruti, I. Chaudieu, M. Chicheportiche, J. M. Kamenka and R. Chicheportiche. Ecole Nationale Supérieure de Chimie, CNRS L.P. 8402, INSERM U 249, 8 Rue de l'Ecole Normale, 34075 Montpellier Cédex, France.

Phencyclidine (PCP) and analogs are known to inhibit the dopamine (DA) uptake by nerve endings. It has been shown that this effect may be correlated to their binding properties to the PCP receptor. These results have been extended to a larger number of molecules (n=37). It appears from these experiments that there exist two different classes of PCP analogs. Molecules which possess an intact phenyl ring verify very significantly the correlation (r=0.97, F=180, slope=0.95 for 14 molecules). Substitution of the phenyl ring or incorporation of a 2-thienyl ring instead of the phenyl lead to a loss of the correlation. Thus it appears from these results that an aromatic ring is required for the binding to the DA uptake system and the PCP receptor and that this aromatic component of the molecule must be a phenyl ring for the validation of the correlation. One of these molecules, GK13, which possesses a benzothiophene ring instead of the phenyl ring exhibit a very high affinity for the DA uptake system (7 nM) and thus appears as one of the most active molecule at this level. GK13 has a low affinity for the PCP receptor (830 nM). This lead us to study the binding properties of tritium labeled GK13 ([$^3$H]GK13) to striatal membrane preparations. The binding is sodium dependent and analysis of data reveals two binding sites for the molecule. One of very high affinity (Kd=0.9 nM, Bmax=3.5–5 pmol/mg protein) and a second one of lower affinity (Kd=20 nM; Bmax=7–10 pmol/mg protein). Furthermore competition experiments with different classes of molecules have shown that [$^3$H]GK13 labels the DA uptake system. The binding site of [$^3$H]GK13 appears different from the PCP receptor since on the same preparation [$^3$H]PCP binding is not sodium dependent and its maximum number of binding sites is only of 0.71 pmol/mg protein.

## STUDIES OF THE HALOPERIDOL SENSITIVE SIGMA RECEPTOR USING NOVEL PROBES DERIVED FROM THE SELECTIVE LIGAND DTG. Weber,* E. and J. F. W. Keana. *Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201; and Department of Chemistry, University of Oregon, Eugene, OR 97403.

We have recently characterized a novel, highly selective ligand [1,3-di-ortho-tolyl-guanidine(DTG)] for the haloperidol-sensitive sigma receptor. We will present the synthesis and characterization of up to 20 different analogs of this compound. These structure-activity studies have allowed us to draw conclusions regarding some of the properties that confer sigma-receptor affinity and selectivity to DTG-related compounds. Knowledge from these structure/activity studies has also allowed us to synthesize a novel irreversible ligand for the haloperidol sensitive sigma receptor. Some of the receptor binding characteristics of this novel affinity

label for sigma receptors will be presented. Finally we will present methods that allow solubilization of active sigma receptors and we will show that the novel probes described above are useful tools for characterizing the solubilized binding sites.

PHENCYCLIDINE DISCRIMINATION AND N-METHYL-D-ASPARTATE RECEPTOR STIMULATION AND ANTAGONISM IN RATS. Willetts, J. and R. L. Balster. Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Much evidence exists to suggest that phencyclidine (PCP) acts to inhibit excitatory amino acid (EAA) neurotransmission mediated by N-methyl-D-aspartate (NMDA)-sensitive EAA receptors: PCP and PCP-like drugs selectively antagonize NMDA-induced excitation of central neurons, PCP prevents NMDA-induced transmitter release and binding studies show that while PCP and NMDA receptor are distinct, they may be intimately associated. Evidence also suggests there is some overlap in the behavioral effects of PCP and NMDA-receptor blocking drugs (Koek *et al., Behav Brain Res* **19:** 257–259, 1986; Koek *et al., Life Sci* **39:** 973–978, 1986). We, therefore, decided to investigate whether the NMDA antagonist 2-amino-7-phosphonoheptanoate (APH) would produce PCP-like discriminative stimulus effects and whether NMDA could block the discriminative stimulus properties of PCP in rats. Male Sprague-Dawley rats were trained to discriminate between PCP (1.25 mg/kg IP) and saline on a two lever fixed-ratio 32 schedule of food reinforcement. During the last 10-minute segment of a 30-minute test session, intracerebroventricular (ICV, 0.375–30.0 $\mu$g), but not systemic (10–30 mg/kg IP), administration of APH produced greater than 80% PCP-lever responding in eight out of ten rats, though the dosage at which generalization occurred varied between rats. NMDA (0.05–0.30 $\mu$g ICV) was administered 10 minutes after PCP and immediately before test sessions. While the higher doses of NMDA produced modest reductions in PCP-lever responding during the first 10-minute segment of the 30-minute sessions, these were usually accompanied by large reductions in response rates. Brief convulsions also followed administration of the highest dose of NMDA. Thus, APH shares some discriminative stimulus properties with PCP in rats. However, since PCP-lever selection was not as consistent as it was following PCP administration, there may not be a complete overlap in the discriminative stimulus properties of PCP and APH. We also conclude that ICV administration of NMDA does not antagonize the discriminative stimulus properties of PCP. These results are in agreement with those of Leander *et al. (Excitatory Amino Acid Neurotransmission.* New York: Alan R. Liss Inc., 1987, pp. 197–204) who observed that NMDA does not antagonize behavioral suppressant effects of PCP in pigeons. (Research supported by NIDA Grant DA-01442.)

BEHAVIORAL SIMILARITY OF DI-ORTHO-TOLYL-GUANIDINE (DTG), A SELECTIVE LIGAND FOR A HALOPERIDOL-SENSITIVE SIGMA BINDING SITE, TO PHENCYCLIDINE (PCP) IN RATS, PIGEONS, AND RHESUS MONKEYS. Woods, J. H., W. Koek and E. Weber. University of Michigan, Ann Arbor, MI 48109; and Oregon Health Science University, Portland, OR 97201.

DTG is a highly potent ligand for a binding site in rat brain that also recognizes certain PCP-like substances, haloperidol, and 3-(3-hydroxy)-phenyl-N-propylpiperidine (Weber *et al., Proc Natl Acad Sci USA,* in press). An advantage of this ligand is its high affinity and selectivity for this site, when compared with the affinity and the selectivity of the other aforementioned compounds. DTG was compared to PCP in a number of behavioral preparations. In pigeons, intramuscular administration of DTG up to lethal doses failed to induce a PCP-like catalepsy. PCP-like discriminative stimulus effects in pigeons and in rhesus monkeys were neither induced nor potentiated or antagonized by behaviorally active doses of DTG. Up to lethal systemic doses, DTG failed to produce directly observable PCP-like behavioral effects (e.g., locomotion, sniffing, swaying and falling) in rats. In addition, while producing ataxia, DTG failed to produce PCP-type anesthesia, characterized by absence of eye closure and muscle relaxation, in rhesus monkeys. Thus, our preliminary data suggest that DTG does not share behavioral effects with PCP. DTG (10–10 mg/rat) when given intraventricularly in rats produces a dose-dependent forelimb extension and backward walking; perhaps, resembling some psychotomimetic opioids. The further characterization of behavioral effects unique to DTG is important to assess the relevance of its binding site. (Supported in part by USPHS Grant DA-00154.)

REGIONAL LOCALIZATION OF NMDA AND TCP BINDING IN MAMMALIAN BRAIN. Young, A. B., W. F. Maragos and J. B. Penney. Department of Neurology, University of Michigan, Ann Arbor, MI 48104.

Electrophysiological, behavioral and pharmacological studies suggest that dissociative anesthetics block the excitatory actions of N-methyl-D-aspartate (NMDA) in the CNS through an allosteric interaction at the channel linked to the NMDA receptor. Such an interaction would predict that the two receptors have identical regional distributions in brain and should be present in some fixed ratio. We have measured TCP and NMDA receptors in serial sections of rat and human brain using [3H]N-(1-[2-thienyl]cyclohexyl) 3,4-piperidine (TCP) and [3H]glutamate as previously described (Maragos *et al., Eur J Pharmacol* **123:** 173–174, 1986). There was a marked correlation between the regional localization of NMDA receptors and TCP receptors in rat and human brain. In 52 regions of rat brain, NMDA receptor density correlated with TCP receptor density ($r=0.95$; $p<0.001$). Binding was highest in stratum radiatum of hippocampus.