

STUDIES ON INHIBITION OF PHENCYCLIDINE-INDUCED HYPERACTIVITY BY GLYCINE. Toth, E. Center for Neurochemistry; Nathan, S. Kline Institute for Psychiatric Research, Ward's Island, New York, NY 10035.

We found recently that glycine inhibits phencyclidine (PCP)-induced hyperactivity without affecting the cerebral uptake of PCP in mice (Toth, E. and A. Lajtha, *Neurochem Res* 11: 393-400, 1986). Since the latter is an indication of central effect, the possible mechanism of the inhibitory action of glycine was investigated. A single dose of glycine (50 $\mu\text{mol/g}$) that suppressed 75% of the hyperactivity induced by 5 $\mu\text{g/g}$ PCP seemed to have no effect on the metabolism and binding of PCP. The amount of chloroform-extractable and particulate-bound radioactivity of the whole brain homogenates was similar in the glycine treated animals to that of controls after 10 min and 20 min following the administration of ^3H -PCP. The glycine had no significant effect on regional distribution of the drug in olfactory bulb, frontal cortex, lateral cortex, midbrain, pons-medulla, and cerebellum. It also did not affect the cerebral metabolism and the binding of PCP *in vivo*. Glycine affected the subcellular distribution of PCP. There was a reduction in radioactivity following the injection of ^3H -PCP (0.02 $\mu\text{Ci/g}$) in the mitochondrial and microsomal fractions of the whole mouse brains by 30% and 40% respectively. The inhibitory effect of chlorpromazine on PCP-induced hyperactivity was greatly enhanced by glycine. Following the glycine treatment of mice, there was an increase in cerebral glycine and glutamine (100% and 80%) and a decrease in aspartate, glutamate, and taurine (10-20%). PCP (5 $\mu\text{g/g}$) had no effect on the level of neurotransmitter amino acids. The binding of spiroperidol *in vivo* was reduced 50% by glycine in the brains. It is suggested that the mechanism of inhibition of PCP-induced hyperactivity by glycine involves: (1) alteration in level of amino acid neurotransmitters, (2) alteration in the subcellular distribution of PCP, and (3) effect on the central dopaminergic system. It is possible that glutamine mediates the inhibitory action of glycine since both chlorpromazine and glycine increase cerebral glutamine levels and enhance each other's inhibitory action on PCP-induced hyperactivity in mice.

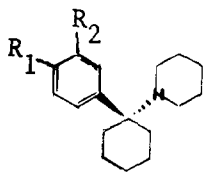
PHENCYCLIDINE METABOLISM: INVOLVEMENT ON IMINIUM ION INTERMEDIATES IN COVALENT BINDING, SUICIDE INACTIVATION OF CYTOCHROME P-450 AND FORMATION OF A NOVEL METABOLITE. Trevor, A. J., M. P. K. Hoag, M. Schmidt-Peetz and N. Castagnoli, Jr. Division of Toxicology, Departments of Pharmacology and Pharmacy, University of California, San Francisco, CA 94143.

The 1-(1-phenylcyclohexyl)-2,3,4,5-tetrahydropyridinium species (PCP-Im⁺) is a major metabolite formed from phencyclidine (PCP) during its cytochrome P-450-dependent oxidation by liver tissues. Nucleophilic "trapping" of PCP-Im⁺ with cyanide ion prevents the metabolism-dependent covalent binding of radiolabelled PCP to liver macromolecules and the mechanism-based inactivation of cytochrome P-450 by PCP. Synthetic PCP-Im⁺ added to liver microsomes inactivates cytochrome P-450 and the radiolabelled compound binds irreversibly to microsomal components. Both processes require further metabolism of the intermediate. The

mechanism-based inactivation of cytochrome P-450 by PCP and PCP-Im⁺ is enhanced by pretreatment of rodents with phenobarbital. Selectivity of this inactivation for phenobarbital-inducible isozymes of cytochrome P-450 has been shown using purified forms of the enzyme (M. Coon *et al.*). Incubation of PCP-Im⁺ with liver microsomes in air plus NADPH has led to the isolation and characterization of 1-(1-phenylcyclohexyl)-2,3-dihydro-4-pyridone as a primary metabolite, the structure of which was confirmed by synthesis. This 4-electron oxidation product of PCP-Im⁺ is likely to occur via formation of a 2-electron intermediate, possibly the allylic alcohol, which also would be expected to undergo spontaneous dehydration to form reactive dihydropyridinium species. These metabolic transformations will be discussed in terms of the bioactivation of PCP to potentially toxic products. (Supported in part by NIDA grant DA 3405.)

^{18}F -PCP ANALOGS FOR POSITRON EMISSION TOMOGRAPHY (PET). Van Dort, M. E., D. J. Yang, M. R. Kilbourn, D. J. Gole, A. Kalir, D. C. Chu, A. B. Young, E. F. Domino and D. M. Wieland. University of Michigan Medical Center, Ann Arbor, MI 48109; and Tel Aviv University, Tel Aviv, Israel.

Glutamate receptors have been classified into various, subtypes of which the N-methyl D-aspartate (NMDA) receptor is of special interest. Drugs such as phencyclidine (PCP) and a PCP analog TCP bind either to an allosteric NMDA site or possibly to the ion channel itself. These recent findings have created a renewed interest to find a PCP-like compound for mapping NMDA-linked glutamate receptors in the human brain by PET. ^{18}F - and ^{11}C -labeled analogs of PCP are being investigated in our laboratories as possible probes for imaging NMDA type glutamate receptors in brain. Structural modifications by the introduction of substituents on either the cyclohexyl, piperidine or phenyl rings of PCP (1) leads to compounds with varying degrees of PCP-like activity. The relative potencies of some of these PCP analogs were determined *in vivo* in mice using motor coordination assessed by the platform test. In general, it was observed that substitution of either the cyclohexyl or piperidine rings resulted in a compound having lower PCP-like activity, probably due to a lower affinity of these molecules to receptor sites. Substitution on the phenyl ring either decreased or increased PCP-like behavioral activity depending upon the nature of the substituent and its position on the phenyl ring. The ED_{50} of PCP given IP in the mouse platform test was 19.7 $\mu\text{mol/kg}$. Substitution of either -OH or -NH₂ at the *m* position on the phenyl ring resulted in compounds with high potency and substitution of halogens in the *p* position resulted in compounds with relatively low potency. The ED_{50} s of *m*-OH, *m*-NH₂ (2) and *p*-Cl were 7.66 $\mu\text{mol/kg}$, 4.55 $\mu\text{mol/kg}$ and 26.8 $\mu\text{mol/kg}$, respectively. Compound 3 with a -F atom in the *p* and -NH₂ group in the *m* position still retained its high potency (ED_{50} 4.48 $\mu\text{mol/kg}$). Using ^3H -TCP and rat brain homogenate, competitive binding studies are in progress with compounds 1-3. Preliminary studies have shown that reaction of 4 with ^{18}F -fluoride in DMSO at 160°C provides the respective ^{18}F analog 5 in an approximately 15% radiochemical yield as determined by radio-HPLC. Rapid reduction of 5 to 6 and further optimization of the ^{18}F -labeling reaction are under study.



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 alpha₁-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 alpha₁-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 μM) 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 stimulatory 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 [³H]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 ([³H]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 (K_d=0.9 nM, B_{max}=3.5–5 pmol/mg protein) and a second one of lower affinity (K_d=20 nM; B_{max}=7–10 pmol/mg protein). Furthermore competition experiments with different classes of molecules have shown that [³H]GK13 labels the DA uptake system. The binding site of [³H]GK13 appears different from the PCP receptor since on the same preparation [³H]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