ELECTROPHYSIOLOGICAL STUDIES OF THE INTERACTION BETWEEN PHENCYCLIDINE/*SIGMA* RECEPTOR AGONISTS AND EXCITATORY AMINO ACID NEUROTRANSMISSION ON CENTRAL MAMMALIAN NEURONES.

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Since the original observations that low doses of ketamine, phencyclidine (PCP), etoxadrol and cyclazocine were selective antagonists of N-methyl-D-aspartate (NMA) on spinal neurones were reported at the 1st of these French-U.S. seminars in Montpellier 1982, much work has gone into attempting to ascribe some of the biochemical and behavioural properties of PCP-like drugs to reduced excitatory transmission. In the spinal cord, cerebral cortex and other parts of the CNS, synaptic excitations which on other grounds are thought to be mediated by NMA receptors but not those mediated by other receptors are also reduced by *sigma* opiates and dissociative anaesthetics. Using hemisected frog spinal cords and wedges of cerebral cortex, we have estimated the IC_{50}s of a series of such drugs to reduce neuronal depolarisation by NMA. Their potency as NMA antagonists appears to correlate with their ability to displace PCP binding, to mimic PCP in drug discrimination studies, to prevent epileptiform activity, to reduce glutamate-induced calcium uptake into brain slices and to limit the neuronal damage that follows hypoxia *in vitro* and ischaemia *in vivo*. Synaptosomal release of rubidium, a measure of potassium conductance, is inhibited by these and related drugs in a manner that does not correspond to PCP receptor activity. Furthermore, the fact that PCP-like drugs do not increase central synaptic transmission argues against them causing an enhanced presynaptic release of neurotransmitter. NMA antagonism by PCP-like compounds is not competitive. The observation of a dependency on exposure of *in vitro* preparations to NMA agonists in order to fully develop the block of their action by some of the PCP-like compounds as well as the voltage-dependency of this antagonism reported elsewhere suggest that the PCP/*sigma* receptor may be located in the channel opened by activation of the NMA receptor. If this concept of a channel plugging action of PCP proves correct, then it seems unlikely that a PCP receptor antagonist will be found. If on the other hand PCP proves to modulate the activation of the NMA receptor channel in a manner akin to that of the benzodiazepine-GABA interaction then PCP antagonists should be found. If our hypothesis that NMA antagonism explains some of the behavioural properties of PCP, it should be possible to mimic or ameliorate the action of PCP by pharmacological modulation, reduction or facilitation respectively, of synaptic excitation mediated by NMA receptors. (Supported by the MRC and the Wellcome Trust.)

DIFFERENT PATTERNS OF CEREBRAL GLUCOSE UTILIZATION PRODUCED BY PHENCYCLIDINE AND (+)N-ALLYLNORMETAZOCINE.

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Phencyclidine (PCP) and N-allylnormetazocine (NANM) share several properties, including the abilities to produce psychotomimetic effects, to be self-administered and to bind at specific cerebral sites. Because of interest in the mechanisms which mediate the behavioral effects of PCP and *sigma* agonists, we studied the effects of PCP (0.5, 1, 5, 10 mg/kg) and (+)NANM HCl (5 mg/kg) on rates of local cerebral glucose utilization (LCGU) in awake rats, by the autoradiographic 2-deoxy-D-[1-^{14}C]glucose method (L. Sokoloff *et al.*, *J Neurochem* **28:** 897, 1977). This method has been used to demonstrate a close relation between cerebral function and glucose utilization, and has been helpful in identifying brain areas affected by various drugs *in vivo* (J. McCulloch, in: *Handbook of Psychopharmacology*, vol 15, edited by L. L. Iversen *et al.*, New York: Plenum, p. 321, 1982). Rats received an IV injection of PCP, NANM, 2 or 15 min before the radiotracer, respectively, or 0.9% NaCl at corresponding times, and LCGU was determined as described by Sokoloff *et al.* (1977). PCP produced stereotypies, ataxia, and various effects on LCGU, which varied with dose. LCGU increased throughout the limbic system, except the habenula. LCGU increased in most sensory structures, but decreased in specific layers of the somatosensory and auditory cortices and the inferior colliculus. Responses of specific thalamic relay areas appeared to be dissociated from activity in their terminal fields in the cortex. LCGU increased throughout the motor system, showing a striking pattern of columnar activity in the motor cortex. However, LCGU was reduced in the frontal cortical pole. Rats given injections of NANM also showed ataxia and stereotypies, but the quality and distribution of the effects of (+)NANM on LCGU differed widely from those of PCP. LCGU was generally reduced, with statistically significant effects in the cerebellum, superficial layers of the visual and auditory cortices, the superior colliculus and its thalamic projection, the lateral posterior thalamic nucleus. NANM also decreased LCGU in the superficial layers and layer 4 of the motor cortex and in the superficial layers of the frontal pole. The dorsal hippocampus showed a reduction of LCGU in CA1. Significant decreases also were seen in the habenula. Differences between the anatomical distribution of PCP- and (+)NANM-induced effects on LCGU are consistent with the differential localizations of haloperidol-sensitive *sigma* (σ) and PCP receptors in the rat brain (B. L. Largent *et al.*, *J Pharmacol Exp Ther* **238:** 739, 1986). Further studies with specific agonists and antagonists may help clarify a neuroanatomical basis for specific behaviors produced by interactions at PCP and σ receptors.

1-(1-ALKYNYLCYCLOHEXYL) PIPERIDINES (ACETYLENIC ANALOGS OF PHENCYCLIDINE).

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Phencyclidine analogs bearing an alkynyl or alkyl substituent instead of phenyl, were prepared and their physiological and pharmacological activity tested with the following results: (a) the hydrophobic properties of the substituent contribute to the antiacetylcholine activity of the drug at least as much as the electron density of unsaturated bonds, (b) the degree of central activity in intact animals is considerably reduced when the phenyl group is replaced by an alkyl (saturated or unsaturated) with a maximum activity for a three-carbon chain, (c) the general effects elicited in higher mammals resemble those produced by phencyclidine al-

though to a much lesser extent, (d) the propargyl derivative, (*4*, R=CH=CH_2–) seems to evoke in monkeys a "hallucinogenic effect" similar to that of phencyclidine.

EFFECTS OF SELECTIVE CORTICAL AND SUBCORTICAL LESIONS ON TCP AND NMDA RECEPTOR BINDING. Maragos, W. F., D. C. M. Chu, A. B. Young and J. B. Penney. Department of Neurology, University of Michigan, Ann Arbor, MI 48104.

The dissociative anesthetics (phencyclidine, ketamine, and N-(1-[2-thienyl]cyclohexyl])-piperidine (TCP) are a unique class of drugs which appear to interact with a site closely linked to the channel of the N-methyl-D-aspartate (NMDA) receptor. While much data has recently been gathered concerning the pharmacological, electrophysiological and behavioral interactions between these two sites, little is known about their neuronal localization. We have studied both sites autoradiographically in serial sections of rat brains after specific lesions. Male Sprague-Dawley rats (200 g) were anesthetized and lesioned in the (1) nucleus basalis using ibotenate, (2) cerebral cortex using ibotenate, (3) entorhinal cortex using knife cuts and (4) dentate gyrus using colchicine. After one week, animals were decapitated, the brains frozen and samples taken for CAT activity and histological examination. Serial sections were assayed for TCP and NMDA receptors using [3H]TCP and [3H]glutamate as previously described (Maragos *et al.*, *Eur J Pharmacol* **123**: 173–174, 1986). After nucleus basalis lesions neither binding site was altered in cortex. Entorhinal knife cuts resulted in only a 9% decrease in dentate TCP binding. Cortex and dentate lesions caused local 94–98% and 84–92% reductions in both receptor sites, respectively. The data suggest that NMDA and TCP receptors have similar localizations and that for the most part, they are localized postsynaptically. (Supported by USPHS grants AG 06155 and NS19613 and the ADRDA.)

KETAMINE PREVENTS GLUTAMATE-INDUCED CALCIUM INFLUX AND ISCHEMIC NERVE CELL INJURY. Marcoux, F. W., J. E. Goodrich, A. W. Probert, Jr. and M. A. Dominick. Departments of Pharmacology and Toxicology and Experimental Pathology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Ketamine has been reported to block glutamate and hypoxia-induced neuronal injury in culture. Glutamate's excitotoxic effects have been proposed to be the result of intracellular calcium accumulation via NMDA receptor-coupled channels. We examined the effects of ketamine against glutamate-induced calcium influx into cultured neurons from the rat cerebral cortex and against ischemic neuronal injury in vivo in the gerbil hippocampus. Cells were harvested from neonatal cerebral cortex, cultured in 96-well plates (2×10^5 cells per cm^2) for 8–14 days, preincubated for 30 min in $^{45}C^{++}$ and then challenged for 30 min with 100 μM glutamate with or without (+)-ketamine. After wash, intracellular $^{45}Ca^{++}$ concentration was measured for individual wells in a scintillation counter. Ketamine's effect on ischemic CA1 neuronal injury were assessed using a temporary bilateral carotid occlusion model in gerbils. The effects of Ketalar®(ketamine hydrochloride) pretreatments were assessed on global ischemia-induced increases in exploratory locomotor activity and depletion of hippocampal pyramidal neurons. One hundred μM glutamate-induced $^{45}Ca^{++}$ influx into cultured neurons was assessed on control wells and in the presence of ketamine at 0.001 to 1000 μM concentrations. At 250 μM, ketamine inhibited glutamate-induced $^{45}Ca^{++}$ influx by 90%; the IC_{50} was 6.8 μM. Gerbils were administered 100, 150 or 200 mg/kg Ketalar® pretreatments 30 min before 10 min of bilateral carotid occlusion. Exploratory locomotor activity 24 hr later and CA1 hippocampal light microscopic histopathology 2 weeks later were compared with that of vehicle pretreated control gerbils. There were dose-related attenuations in the hyperlocomotor activity responses of the Ketalar® vs. control gerbils; the anesthetic 200 mg/kg Ketalar® dose prevented an increase in locomotor activity. In the same Ketalar® vs. control gerbils there were dose-related reductions in CA1 neuronal depletion as judged by a blinded histopathological evaluation. Five out of 12 gerbils given the 200 mg/kg Ketalar® pretreatment showed no CA1 neuronal depletion. These results support a glutamate antagonist action for ketamine. In addition, the findings with gerbils suggest that Ketalar® anesthesia protects against ischemic neuronal injury. Further studies may provide evidence that ketamine's inhibition of glutamate-induced calcium influx is responsible for its apparent neuroprotective action as shown by the present results.

PRIMARY CULTURE OF MONOAMINERGIC NEURONS AS A MODEL OF THE SCREENING OF PCP DERIVATIVES. Marlier, L., M. J. Drian, J. M. Kamenka* and A. Privat. Neurobiologie du developpement, INSERM U-249, CNRS LP 8402 and *Laboratoire de Biochimie Générale ENSCM Montpellier, France.

The brainstem of 13–14 days rat foetuses was dissected in order to prepare cell suspensions containing either serotonergic or dopaminergic or noradrenergic neurons. These suspensions were plated on multiwell dishes coated with polylysin and cultivated in a semi-synthetic medium for periods up to two weeks. Monoaminergic neurons were immunocytochemically identified with specific antibodies against serotonin, dopamine and noradrenaline. Specific uptake of the corresponding neurotransmitters was controlled with radioautography, and found to coincide with immunocytochemical label. The ability of a new PCP derivative, GK 13, to inhibit specific uptake of monoamines was assayed on the three culture models. It was found that the drug inhibited 50% of the uptake at a concentration of 0.7 10^{-6} M for serotonin, 0.1 10^{-6} M for dopamine and noradrenaline. Moreover, when compared with nomifensin, GK 13 was found much more potent as an inhibitor of dopamine uptake. These results will be compared with those obtained with PCP and other derivatives. These data suggest that primary cultures of identified monoaminergic neurons are a useful tool for the study of the characteristics of PCP and its derivatives. (Supported by INSERM and CNRS.)