reached in the cultures receiving enhancing serum before or at the same time as the peak RT activity of control infections. Moreover, we have shown in other experiments that RT activity mirrors viral antigen (p25) as well as the amount of infectious virus production (*10*). Thus, HIV enhancement is characterized not only by accelerated kinetics of viral replication but also by increased virus progeny.

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Pathological Changes Induced in Cerebrocortical Neurons by Phencyclidine and Related Drugs

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Phencyclidine (PCP), a dissociative anesthetic and widely abused psychotomimetic drug, and MK-801, a potent PCP receptor ligand, have neuroprotective properties stemming from their ability to antagonize the excitotoxic actions of endogenous excitatory amino acids such as glutamate and aspartate. There is growing interest in the potential application of these compounds in the treatment of neurological disorders. However, there is an apparent neurotoxic effect of PCP and related agents (MK-801, tiletamine, and ketamine), which has heretofore been overlooked: these drugs induce acute pathomorphological changes in specific populations of brain neurons when administered subcutaneously to adult rats in relatively low doses. These findings raise new questions regarding the safety of these agents in the clinical management of neurodegenerative diseases and reinforce concerns about the potential risks associated with illicit use of PCP.

THE DRUG PCP, A DISSOCIATIVE ANesthetic, is best known as an abused street drug with potent psychotomimetic properties. PCP binds with high affinity and specificity to a unique class of membrane receptors in the mammalian central nervous system (CNS) (*1*), suggesting that the CNS may contain a yet to be identified PCP-like peptidergic neuromodulator and that dysfunction of this neuromodulatory system might underlie psychotic disorders such as schizophrenia (*2*). PCP receptors are colocalized with *N*-methyl-D-aspartate (NMDA) receptors (a subtype of glutamate receptor) (*3*), and PCP antagonizes NMDA receptor–mediated neuroexcitatory (*4*) and neurotoxic (*5*) phenomena. MK-801 is a PCP-like compound that displays even greater potency than PCP in binding to the PCP receptor and in antagonizing the excitatory (*6*) and toxic (*7*) actions of NMDA. MK-801 or PCP can protect CNS neurons against hypoxic-ischemic, hypoglycemic, or epilepsy-related brain damage (all of which are postulated to be NMDA receptor–mediated processes) (*8, 9*). Here we report that, in addition to their potent neuroprotective properties, these agents induce pathomorphological changes in certain CNS neuronal populations when administered subcutaneously to adult rats.

Treatment of rats with kainic acid causes persistent limbic seizures that result in a distinctive pattern of seizure-related brain damage (*10*). We have observed that PCP and MK-801 protect against this type of damage (*11*). However, the protected brains, although appearing normal in regions typically vulnerable to seizure-related brain damage, displayed neuropathological changes (vacuolization of neuronal cytoplasm) in the posterior cingulate and retrosplenial neocortices, changes that are subtly different from those typically associated with kainic acid treatment. Examination of the brains of control animals treated only with MK-801 or PCP revealed these same changes in cingulate and retrosplenial neurons, even though these animals had not been exposed to any convulsant and had not experienced any seizures. No such changes could be found in control animals that received no drug treatments. Because the vehicle was water (*12*), it could not have caused the changes. Therefore, we evaluated the possibility that PCP and MK-801 might have cytotoxic effects on CNS neurons.

Adult, female Sprague Dawley rats (300 g) were injected subcutaneously (sc) with an aqueous solution (*12*) of MK-801 (0.05 to 1.0 mg per kilogram of body weight, sc) or PCP (0.5 to 5.0 mg/kg sc) and were killed 4 hours later for histopathological evaluation of the brains by light and electron microscopy (*13*). Both compounds caused a dose-dependent vacuolar reaction detectable by light microscopy in cingulate and retrosplenial neurons, the ED_{50} (*14*) being 0.18 mg/kg (0.12 to 0.24) (n = 36) for MK-801 and 2.83 mg/kg (1.72 to 3.93) (n = 36) for PCP. Adult, male Sprague Dawley rats (450 g) were also susceptible but at a slightly higher dose, ED_{50} being 0.32 mg/kg (0.25 to 0.39) (n = 24) for MK-801 and 4.29 mg/kg (2.87 to 5.70) (n = 20) for PCP.

Electron microscopic evaluation of the affected neurons 2 to 4 hours after PCP or MK-801 treatment corroborated that the changes consisted of the formation of multiple vacuoles of heterogeneous size occupying the cytoplasmic compartment (Fig. 1, A and B). At 2 hours, when vacuoles first became evident, it appeared that they were forming from saccules of endoplasmic reticulum, that mitochondria or other cytoplasmic components were being incorporated within them, and that the incorporated structures were undergoing a process of dissolution. At 4 hours, the cytoplasmic compartment of an affected neuron appeared to be packed with vacuoles and devoid of mitochondria. The vacuoles varied in diameter from 3 to 15 μm. A time course study by light microscopy revealed that the vacuoles were detectable 2 hours after a

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single exposure to MK-801 (1.0 mg/kg sc) and became increasingly more conspicuous at 4, 8, and 12 hours. However, in the interval from 18 to 24 hours, the reaction subsided and the morphology of most neurons, as evaluated by light microscopy, appeared to have returned to normal.

We next evaluated the effects of repeated exposure to PCP and MK-801. Rats were treated with MK-801 daily for 4 days at 0.3 mg/kg sc and then killed, and the brains were evaluated by light microscopy 4 hours after treatment on day 4. The brains of animals subjected to this treatment regimen displayed no morphological changes. When a gradually increasing dose schedule (0.2, 0.4, 0.6, and 0.8 mg/kg sc) was used and treatments were spaced 12 hours apart, no cytopathology was seen 4 hours after the last treatment. When rats were treated daily with MK-801 for 4 days with higher or more steeply increasing dose schedules (0.25, 0.5, 0.75, and 1.0, or 0.25, 0.5, 1.0, and 2.0 mg/kg sc on days 1, 2, 3, and 4, respectively) and the brains were examined 4 hours after the treatment on day 4, these brains displayed the typical cytopathological changes that would be expected 4 hours after a single treatment, but there was no evidence (by light microscopy) of a cumulative effect or of the reaction progressing to an irreversible stage. A single treatment with MK-801 (0.5 mg/kg sc) followed by a day without drug, then renewed treatment (MK-801, 0.5 mg/kg sc) on day 3 resulted in the typical pathomorphological reaction 4 hours after the treatment on day 3. Experiments of similar design were conducted with PCP and the same results were obtained, suggesting apparent tolerance.

Rats treated with very high doses of MK-801 (5 or 10 mg/kg sc) and examined 24 to 48 hours later displayed vacuolar changes in cingulate and retrosplenial cortical neurons at both time intervals. Thus far, only light microscopic evaluation of changes at these high doses has been performed.

On the basis of a relatively comprehensive, although not exhaustive, light microscopic evaluation of the brains of rats treated with PCP or MK-801 to determine how many CNS neuronal populations are susceptible to the apparent toxic effects of these agents, we believe that this action may be quite selective for specific neurons in the posterior cingulate and retrosplenial cortices. The affected neurons are medium to large in size and multipolar or pyramidal in shape and are located in cortical layers III and IV (Fig. 1C).

To further clarify the role of PCP receptors, we tested two other PCP receptor ligands, tiletamine and ketamine. Both agents are anesthetics used in veterinary medicine, and ketamine is used in human anesthesia (*15*). Each drug was administered in aqueous solution (*12*) as a single dose (1, 5, 10, and 20 mg/kg sc for tiletamine and 5, 10, 20, and 40 mg/kg sc for ketamine) to adult rats ($n = 6$ per treatment group). Examination of the brains 4 hours later revealed vacuole formation in cingulate and retrosplenial cerebrocortical neurons after tiletamine treatment at 10 and 20 mg/kg and ketamine treatment only at 40 mg/kg. Lower doses of either drug were not associated with cerebrocortical pathological changes.

Our findings suggest that PCP and the related compounds MK-801, tiletamine, and ketamine induce a pathomorphological reaction in the majority of medium- to large-sized neurons in layers III and IV of the posterior cingulate and retrosplenial cortices. The order of potencies with which these agents induced this effect (MK-801 > PCP > tiletamine > ketamine) is the same as their order of affinities for binding to the PCP receptor (*4, 6*) and for their order of potencies in antagonizing either the excitatory (*4, 6*) or neurotoxic (*5, 7*) actions of NMDA. For example, in the isolated chick embryo retina, the concentration of antagonist required to totally prevent the toxic action of 80 μM NMDA is 0.1, 0.5, 2.0, and 5.0 μM, respectively, for MK-801, PCP, tiletamine, and ketamine (*16*). Many ligands for the PCP receptor also bind to the sigma opiate receptor; however, they do so with an order of affinities opposite to the above, and MK-801, the most potent PCP receptor ligand known, has very little affinity for the sigma binding site (*6*). Moreover, the

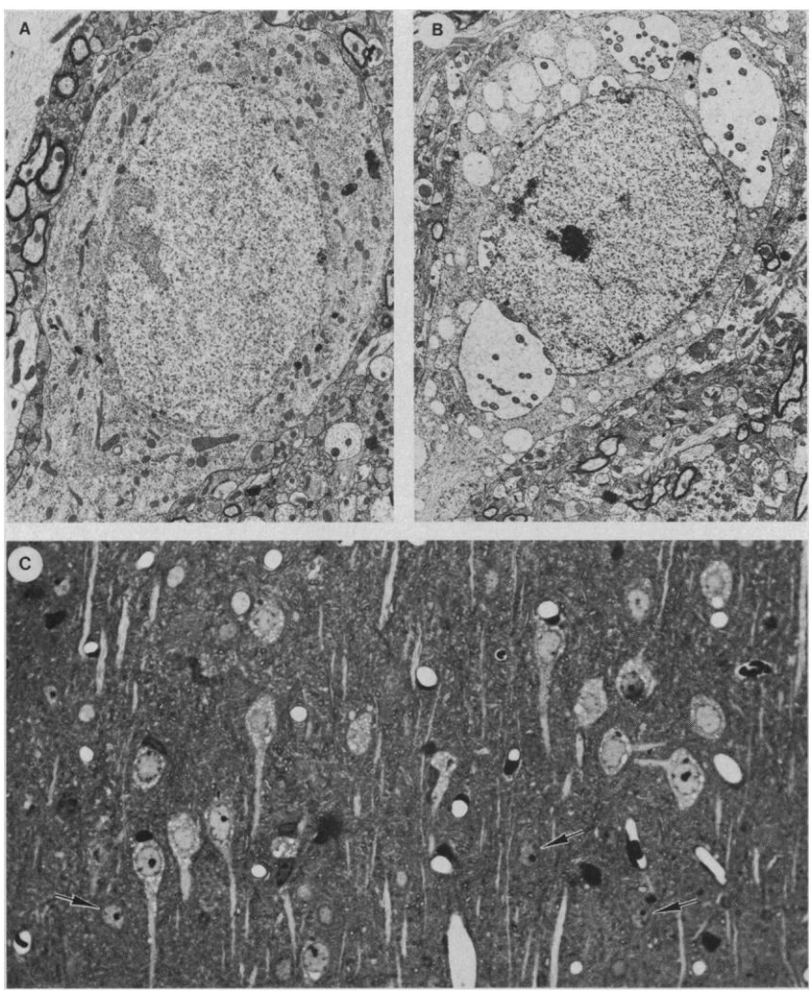

Fig. 1. (**A**) Electron micrograph depicting a large posterior cingulate cortical neuron from the brain of a normal untreated rat. The cytoplasm of this neuron contains many normal-appearing mitochondria, and there are no abnormal vacuoles (×7000). (**B**) A large posterior cingulate cortical neuron from a rat treated with PCP (5 mg/kg sc) 4 hours earlier. Very few normal mitochondria are evident in the cytoplasm but many vacuoles are present, some of which contain multiple small, round structures that appear to be remnants of mitochondria. The neuropil surrounding this neuron is well preserved, and there are many normal-appearing mitochondria in the neuropil components (×7000). (**C**) Numerous vacuole-containing large neurons in layers III and IV of the posterior cingulate cortex of a rat treated 4 hours earlier with MK-801 (1 mg/kg sc). Smaller neurons in other layers (arrows) are free from vacuoles (×200).

dissimilar CNS distribution of sigma and NMDA receptors suggests that they are not colocalized or functionally linked. Thus, our evidence does not support involvement of sigma opiate receptors in the effects of PCP on cingulate cortical neurons but is consistent with involvement of the NMDA-PCP receptor–ion channel complex.

Vacuolization in response to PCP or MK-801 becomes increasingly severe in the first 12 hours and then gradually diminishes in the next 12 hours; repeated treatment does not result in cumulative effects. Rather it appears that the vacuolar reaction, at least at low doses, may be self-limiting and is subject to a mechanism whereby, after a single treatment at a given dose, vulnerable neurons become insensitive to further treatment at that dose, unless the first and subsequent treatments are separated by a significant drug-free interval. This response profile has intriguing features of a tolerance phenomenon. Behavioral tolerance and apparent dependence-withdrawal phenomena have been reported in both monkeys and humans after self-administration of PCP (*17*).

The above description of the time course and apparent reversibility is preliminary and is based only on light microscopic observations. Our evidence pertaining to ultrastructural changes in the rat is limited to an acute time interval (2 to 4 hours) after a single treatment with PCP or MK-801. However, because the changes noted at that time were quite striking, particularly with respect to the apparent lytic degradation of the majority of mitochondria in the cytoplasm of affected neurons (Fig. 1B), an effect detectable only by electron microscopy, we cannot rule out the possibility that other treatment regimens would result in progressive pathomorphological changes detectable only by electron microscopy. We are not aware of reports of neuropathological changes in humans exposed to either PCP or ketamine (*18*).

Because PCP causes schizophrenia-like psychotic symptoms in the human, the finding that specific neurons in the cingulate and retrosplenial cortices are selectively vulnerable to an apparent neurotoxic action of PCP potentially implicates these neurons in the psychotic effects of PCP and, conceivably, in the pathophysiology of schizophrenia; that is, if it is a peculiarity of these neurons, in the human as in the rat, to respond pathologically to PCP receptor stimulation, the pathological response might be expressed as a toxic psychosis when exogenous PCP is the stimulant, or as an endogenous psychosis (schizophrenia) when an endogenous PCP receptor ligand is the stimulant (*2*). It is known that schizophrenics are sometimes remarkably unresponsive to pain (*19*), that a major function of cingulate cortical neurons is to mediate affective responses to pain (*20*), and that dissociative anesthetics (PCP and ketamine) are particularly effective in depressing pain appreciation at thalamocortical levels (*14*). Benes *et al.* (*21*) have described structural abnormalities in the anterior cingulate cortex of individuals with schizophrenia. Whether such changes might also be present in the posterior cingulate or retrosplenial cortices of schizophrenics remains to be determined.

The neuroprotective properties of PCP and MK-801 (*5, 7, 8*) have generated interest in using these agents for therapeutic or prophylactic purposes in various neurodegenerative conditions. A major concern is the possibility that they might induce psychotomimetic side effects. However, it has been reasoned that they might be used to protect against brain damage associated with stroke, cardiac arrest, or perinatal asphyxia because these are acute conditions in which the drug would only need to be given on a one-time basis, and, even if the patient were to suffer a transient psychosis, this might be acceptable in trade for protection against permanent brain damage.

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