Antagonism of phencyclidine action by metaphit in rat cerebellar Purkinje neurons: An electrophysiological study

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ABSTRACT Metaphit (1-(1-(3-isothiocyanatophenyl)cyclohexyl)piperidine), a derivative of the psychotomimetic drug phencyclidine (PCP), is postulated to bind irreversibly to PCP receptors. We examined here the electrophysiological interactions of metaphit with PCP in rat cerebellar cortex, since a specific effect of PCP on cerebral neuronal circuitry has been shown. Metaphit, applied locally to Purkinje neurons by micropressure ejection through multibarreled micropipettes, has a reversible depressant action lasting for 5–20 min. Following this, PCP-induced inhibition is blocked with no recovery despite repeated applications of PCP for over an hour. This blockade was not seen unless the dose of metaphit was sufficient to transiently depress Purkinje neuron discharge. Metaphit does not antagonize inhibitory effects of locally applied norepinephrine or γ-aminobutyric acid. This electrophysiological data suggests that metaphit is an irreversible antagonist of PCP in the cerebellum.

The violently aggressive behavior and long-lasting psychosis induced by PCP [1-(1-phenylcyclohexyl)piperidine] abuse has caused societal concern in many areas of the United States. The psychosis induced by PCP is similar to that displayed in schizophrenia, and it has been noted that it might serve as a useful drug model for schizophrenia. (1) No specific treatment for PCP psychosis is available. (2) For these reasons and many others, the mode of action of PCP in the brain has been investigated by many workers (3).

Extensive evidence has been obtained for the presence of specific receptors in the brain for PCP and similar compounds. Binding sites for which compounds similar to PCP have high affinity have been noted to be most prevalent in the striatum, cortex, and hippocampal regions of the brain. (4–9). There is a high statistical correlation between the binding affinity of these compounds in vitro and their in vivo activity. The stereospecificity of the interaction of compounds similar to PCP with the PCP receptors in vitro and in vivo has also been noted. (9–11). Further, an endogenous ligand for the PCP receptor has been reported. (12). Evidence has been obtained that multiple receptors may exist for compounds similar to PCP (4, 13, 14). Biochemical studies with a new site-directed acylating agent for the PCP receptors have provided further evidence for the existence of multiple receptors for compounds similar to PCP. This affinity ligand, metaphit (1-(1-(3-isothiocyanatophenyl)cyclohexyl)piperidine), irreversibly inactivated only about 50% of the receptors in the striatum and hippocampus, the two brain areas that were investigated. (15). The effect of metaphit on the binding of PCP in striatum has been correlated with a diminished behavioral efficacy of PCP (16).

Our previous findings strongly suggested that stereospecific PCP-induced depressions of cerebellar Purkinje neurons are mediated by catecholamine pathways. (17). The stereospecific effect of the PCP derivative 1-(1-phenylcyclohexyl)-3-methyl-piperidine on cerebellar Purkinje neurons is blocked by antipsychotics in a dose-dependent manner, analogous to the reported blockade of similar norepinephrine (NE) effects in this brain region (18). This antagonism appears to be pharmacologically specific since no antagonism of γ-aminobutyric acid (GABA)-induced inhibitions by neuroleptic drugs was seen in this same study. In addition, the local administration of lithium, which has been shown to block NE effects on cerebellar Purkinje neurons (19), also strongly antagonizes PCP-induced depressions. These noradrenomimetic effects of PCP may be mediated presynaptically in the cerebellum, since local disruption of transmitter release with iotophoretically applied Mg2+ (20) results in decreased PCP efficacy. Also, selective destruction of cerebellar NE-containing afferents with 6-hydroxydopamine (6-OHDA) completely eliminates stereospecific Purkinje cell responses to 1-(1-phenylcyclohexyl)-3-methyl-piperidine.

Because of this previous work and the ease with which Purkinje neurons can be utilized for acute extracellular recordings and local drug administration, we used this target cell for an electrophysiological assessment of the functional significance of metaphit-PCP interactions.

METHODS

Drugs and Materials. The compounds or materials used in the experiments were PCP (R. Hawks, National Institute on Drug Abuse, Rockville, MD), 3H-labeled PCP (New England Nuclear), poly(t-lysine) (Sigma), and Hydrofluor (National Diagnostics, Somerville, NJ).

Synthesis of Metaphit. Metaphit (Fig. 1) was prepared as methanesulfonate and hydrochloride salts. A solution (1.45 ml) of freshly distilled thiophosphogene (2.29 g, 19 mmol) was added to a vigorously stirred two-phase system (4.56 g of 1-[1-(3-aminophenyl)cyclohexyl]piperidine [18 mmol] in 50 ml of chloroform and 756 g of NaHCO3 [90 mmol] in 25 ml of water). The mixture was stirred for 5 min, then the phases were separated, and the aqueous phase was washed once with chloroform. The combined organic layers were dried (over sodium sulfate) and evaporated to a "clear glass" (5.43 g). This substance was dissolved in tetrahydrofuran (20 ml) and treated with a solution of methanesulfonic acid (1.73 g, 18 mmol) in tetrahydrofuran. The resulting crystalline solid was filtered and dried in vacuo to give 7.01 g (98%) of methanesulfonate salt, mp 175–178°C (decomposes). The material could be recrystallized from isopropanol/isopropyl ether (1:2, vol/vol) if necessary. It was stored at 0°C in a

Abbreviations: PCP, phencyclidine; NE, norepinephrine; GABA, γ-aminobutyric acid.
FIG. 1. Structure of metaphit.

desiccator. Spectral data from NMR, chemical ionization mass spectrometry (CIMS), and microanalysis were consistent with the structure shown in Fig. 1. The hydrochloride salt prepared in isopropanol and recrystallized from methanol/ether (1:2, vol/vol) showed mp 214–216°C, as described (15). The methanesulfonate salt was considerably more soluble in water than the hydrochloride.

**Electrophysiology.** Male Sprague-Dawley rats (200–350 g) were anesthetized with urethane (1.25 g/kg intraperitoneally), incubated, and placed in a stereotaxic frame. The skull and dura overlying the cerebellum were removed, and the cerebellar surface was covered with 2.0% agar. The cisterna was opened at the foramen magnum to reduce brain pulsations. Body temperature was monitored with a thermistor probe and maintained at 37°C with a heating pad.

Extracellular action potentials were recorded from single cerebellar Purkinje neurons in lobules VI and VII of the cerebellar vermis through a 5 M NaCl-filled barrel of 3- to 4-barrel micropipettes that were constructed as described (21). This neuronal activity was filtered, monitored on an oscilloscope, and then converted to constant voltage pulses by using a window discriminator. The pulses were integrated over 1-sec epochs by a ratemeter and were displayed on a strip chart recorder. Cerebellar Purkinje neurons were identified by their characteristic discharge pattern of simple and complex spikes.

Drugs were applied from one to three barrels of the micropipette by micropressure ejection as described (21). Pressure applications were regulated by a pneumatic valve (1–35 psi; 1 psi = 6.89 kPa), and the timing of drug pulses was controlled by a crystal clock circuit. Previous studies have shown that drug administration by this technique is reproducible and is linearly related to pressure and time of ejection (22). To be considered valid, all responses to pressure-ejected agonists were required to show reversibility and reproducibility. Controls used to test for local anesthesia, pH effects, drug specificity, and pressure artifacts have been described (30). Drug solutions that were pressure ejected from micropipettes include GABA, 1 mM; NE-HCl, 1 mM; metaphit hydrochloride, 60 μM solution; and PCP, 5 mM. All pressure-ejected drugs were dissolved in 165 mM NaCl.

Ratemeter records were analyzed for changes in neuronal discharge rate caused by local drug application as described (10, 17). The data was digitized and quantified by computer analysis to indicate the percent depression or excitation of neuronal activity caused by drug application. Reports from our laboratory have evaluated the ability of this analytic approach to quantitate responses of neurons to microadministration of drugs (21, 23). Using these techniques, drug responses can be evaluated independently of changes in background discharge, and apparent antagonism due to changes in release of drug from the pipette can be avoided (23). All agonist effects were observed a minimum of six times before and after metaphit application.

**RESULTS**

Application of PCP by micropressure ejection through the multibarreled micropipette reversibly reduced spontaneous discharge rates of cerebellar Purkinje neurons (Fig. 2). PCP generally depressed neuronal discharge after applications of 1–5 sec, with recovery to preapplication discharge rates within 1 min. Metaphit, locally applied to Purkinje neurons

![Fig. 2. Irreversibility of metaphit blockade of PCP-induced responses. A and B represent responses from two different Purkinje cells. The control records (A, record 1; B, record 1) show maximal PCP-induced depressions. After metaphit application (vertical arrow, 5–10 psi, 60 sec) and recovery of discharge, PCP responses are markedly reduced (A, record 2; B, record 2). Repeated PCP applications were continued for 40–60 min with no recovery seen (A, record 3; B, record 3). APS, action potentials per sec.](image-url)
by pressure ejection, also elicited a dose-dependent slowing of spontaneous discharge, but with a much longer latency and time course. It generally took over 1 min to inhibit neuronal discharge. After discharge returned to control levels, 5-20 min later, responses to the depressant actions of PCP were almost completely blocked in all 19 Purkinje cells tested. Generally, PCP blockade was already maximal when spontaneous discharge had recovered from the metaphit application. Sometimes, however, PCP retained a small depressant action after metaphit, which slowly disappeared with continued testing (Fig. 2).

The metaphit-induced blockade of PCP appeared to be irreversible within the constraints of electrophysiological protocols, which require continuous recording from a single neuron. Neurons recorded up to 1 hr after metaphit administration showed no recovery of PCP-induced depression (Fig. 2). The dose of metaphit necessary for these complete and long-lasting antagonisms of PCP was approximately that needed for the initial depressant effects—i.e., unless enough metaphit was applied to cause cessation of firing, little blockade of PCP was subsequently observed.

Specificity of metaphit antagonism of PCP was assessed using two other compounds that inhibit the rat cerebellar Purkinje cell, GABA and NE (23). GABA-induced inhibition of Purkinje cells was unaffected by metaphit in six of seven neurons (Fig. 3). NE-induced slowing was unaltered in 11 of 12 cells (Fig. 4). The absence of change in responses to GABA or NE was observed in neurons in which PCP responses were completely blocked (Figs. 3 and 4).

**DISCUSSION**

This study demonstrates irreversible antagonism of the effects of PCP on Purkinje neuronal discharge by metaphit. A complete antagonism is frequently observed. Interestingly, the antagonism of specific PCP binding was consistently found to be only 50% in striatum and hippocampus (15). The implication is that there are two populations of PCP receptors in these areas, only one of which is affected by metaphit. The present study suggests that only one such receptor, metaphit sensitive, is associated with the effects of PCP in the cerebellum.

In the cerebellum, PCP acts as an indirect noradrenergic agonist (17), so that the PCP receptor sites studied here may be located on noradrenergic nerve terminals, which are afferent to Purkinje neurons. Indirect noradrenergic actions in hippocampus and indirect dopaminergic actions in striatum have also been demonstrated (24, 25). Unlike the cerebellum, however, the striatum and hippocampus also have a significant cholinergic input, where PCP also possesses anticholinergic properties (24). Whether or not these sites are responsible for the fraction of PCP binding in hippocampus and striatum remaining after metaphit treatment is unknown.

PCP is a compound with multiple proposed mechanisms of action, including indirect catecholaminergic effects (17, 25, 26), or opiate effects (27), anticholinergic effects (28), and nonspecific "anesthetic" depressant effects (29). The demonstration of a specific PCP antagonism by metaphit may
provide a useful tool for delineation of the biochemical, physiological, and behavioral actions of this psychotomimetic agent mediated by specific PCP receptors.

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