Village, IL). 5,6-DHT creatinine sulfate was from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was from Hazelton Research Products, Denver, PA. GF/C glass-fiber filters were from Millipore Corp. (Bedford, MA).

Cell Cultures, Incubation Conditions, and Procedures. Starter cultures of neuroblastoma cells, clone N-2a, were kindly provided by Dr. X. O. Breakfield (Yale University School of Medicine). Conditions for growing and differentiating cells were similar to those described earlier[18] except that the medium used was DMEM supplemented with 8% FCS when first plated. All experiments to determine the cytotoxicity and inhibition of [^{3}H]5-HT uptake were conducted on cell suspensions rather than monolayer cultures. All experiments were done with differentiated N-2a cells that were suspended in buffer B by trituration, washed three times with use of a Dynac centrifuge, and resuspended in the same buffer. Cell viability, as determined by trypan blue exclusion, was found to be more than 85%. An aliquot of 10^6 cells per tube, counted by using a hemocytometer, was used for all experiments.

Cytotoxicity of 5,6-DHTs. Cytotoxicity was determined by measuring the inhibition of [^{3}H]thymidine incorporation into the DNA of the N-2a cells. The cells (10^6) were incubated at 37 °C in an incubation mixture of 1 mL of DMEM containing 50, 100, 250, 500, or 1000 μM concentrations of the DHTs. Solutions containing the neurotoxins were made in DMEM immediately before addition to the incubation mixture. The cells were separated by centrifugation and washed with buffer A. Finally, the cell pellets were suspended in 0.5 mL of DMEM containing 50 nM [^{3}H]thymidine (0.5 μCi) and incubated at 37 °C for an additional 90 min. The incubation was terminated by adding 2.5 mL of cold 10% trichloroacetic acid (TCA) and the mixture was kept for 12 h at 0-4 °C. The TCA-precipitated fraction was collected by filtration on GF/C glass fiber filters in a Millipore manifold previously wetted with TCA and washed three times each with 2.5 mL of 95% EtOH. The filter papers containing the precipitates were then carefully transferred to the scintillation vials, dried in an oven at 60-65 °C for 15 min and then digested with 0.5 mL of NCS tissue solubilizer (Amersham) for 30 min. To each sample was added 10 mL of complete counting cocktail, Biosafe II (Research Products International Corp.), and the samples were counted, after overnight storage in the dark, on a Beckman 5801 scintillation counter. Cytotoxicity was expressed (mean ± SD of at least three sets of duplicates) as plots of percent inhibition of [^{3}H]thymidine incorporation with respect to the cells not incubated with the neurotoxins. From these plots (not shown), concentrations required to give 50% inhibition of [^{3}H]thymidine incorporation (IC_{50} values) were calculated by using regression analysis (Table II).

Uptake Affinities. Inhibition of [^{3}H]5-HT uptake was determined by coincubation of cell suspension (10^6) and the respective neurotoxins either at 0-4 °C or at 37 °C for 2 min in an incubation mixture of 1 mL of buffer B containing 0.1 μM [^{3}H]5-HT (4 μCi). Neurotoxin solutions were made in buffer B immediately before addition to the incubation mixture to give a final concentration of 1-100 μM. The uptake experiment was stopped by the addition of 2.5 mL of cold buffer A followed by immediate filtration through GF/C glass fiber filters in a Millipore manifold and washing (5 × 2.5 mL) with the same cold buffer. The filter papers containing the cells were then carefully transferred to the scintillation vials, and the radioactivity was counted as described above. The active uptake is expressed (mean ± SD of at least three sets of duplicates) as the total accumulation of radioactivity at 37 °C minus the accumulation at 0-4 °C (passive diffusion). The degree of potency of the neurotoxins in inhibiting the [^{3}H]5-HT uptake was determined by comparing the concentrations required to give 50% inhibition of active uptake (IC_{50} values) measured at 37 °C. These were calculated by plotting the percentage inhibition of active uptake vs concentration and by using regression analysis (Table III).

Acknowledgment. The support of this work by the National Institute of Neurological and Communicative Disorders and Stroke (Grant NS 15692) is gratefully acknowledged.

Synthesis, Phencyclidine-like Pharmacology, and Antiischemic Potential of Meta-Substituted 1-(1-Phenylcyclohexyl)-1,2,3,6-tetrahydropyridines

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A series of 1-[1-arylcyclohexyl]-1,2,3,6-tetrahydropyridines were prepared by the reaction between 1-(1-cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (1) and an appropriately substituted Grignard reagent. The resulting compounds were tested for their phencyclidine binding site affinities. Selected compounds were then tested for their ability to produce ketamine appropriate responding in monkeys and/or to show neuroprotective effects in a baby rat hypoxia/ischemia model. While it was found that binding site affinity correlated well with discriminative stimulus effects, it was found to be a poor indicator of neuroprotective efficacy within this series.

Recent work by a number of investigators[1-6,11,12] has indicated that compounds with affinity phencyclidine (PCP) binding sites in the central nervous system can exhibit neuroprotective effects in animal models of ischemia. For example, the tricyclic MK-801 has high affinity for PCP binding sites[7] and has been shown to be effective in various animal models of neuroprotection.[8-11] PCP, with a much lower affinity for these sites, exhibits anti-ischemic neuroprotective effects only at much higher

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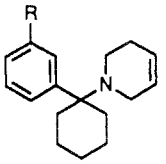
Table I



compd no.	R	K_i for displacement of [^3H]TCP,[a] nM	formula	yield, %	mp, °C	anal.
2a	H	37	$C_{17}H_{23}N$·HCl	61	218–219	C,H,N
2b	OMe	33	$C_{18}H_{25}NO$·HCl	39	209–211	C,H,N
2c	OCH_2Ph	733	$C_{24}H_{29}NO$	76	77–78	C,H,N
2d	OH	7	$C_{17}H_{23}NO$	82	226–228	C,H,N
2e	$CHOCH_2CH_2O$	601	$C_{20}H_{27}NO_2$·HCl	50	161–163	C,H,N
2f	OCH_2OCH_3	92	$C_{19}H_{27}NO_2$·HCl	53	199–201	C,H,N
2g	CHO	324	$C_{18}H_{23}NO$·MsOH	41	211–213	C,H,N
2h	CH=NOH	56	$C_{18}H_{24}N_2O$·HCl	40	233–236	C,H,N
2i	SMe	231	$C_{18}H_{25}NS$·HCl	71	192–195	C,H,N
2j	OAc	6	$C_{19}H_{25}NO_2$·HCl	79	215–217	C,H,N
2k	$CH=CH_2$	114	$C_{19}H_{25}N$·MsOH	58	198–199	C,H,N
2l	F	281	$C_{17}H_{22}NF$·HCl	54	225–226	C,H,N
2m	CH_3	81	$C_{18}H_{25}N$·HCl	72	219	C,H,N
2n	CF_3	422	$C_{18}H_{22}NF_3$·HCl	66	212–217	C,H,N

doses.[12] The neuroprotective effects of PCP-like compounds appear to be highly correlated to their affinity for PCP binding sites. Recent work in our laboratory has been directed toward increasing the binding affinity and improving the neuroprotective effectiveness of the arylcyclohexylamines, the class of compounds structurally related to PCP. Using the PCP binding assay as an indicator of anti-ischemic effectiveness, we sought to modify the structure of PCP in order to identify potentially useful anti-ischemic agents. When structural modifications of PCP are considered it is convenient to consider the in vitro effects of individual changes within each of the three major structural units that comprise the molecule. These are the piperidine, the cyclohexyl, and the aromatic portions of the molecule. It has been demonstrated through work in our laboratory and others, that modifications of the cyclohexyl portion of the molecule, either through substitution, unsaturation, or changes in ring size have been universally detrimental to the binding affinity at the PCP binding site.[13,14] Thus, we chose to focus our efforts upon the other two areas, the piperidine and aryl rings. We had previously found that unsaturation in the 3,4-position of the piperidine ring of PCP gave a substantial degree of enhancement in binding affinity. Similarly it has been shown that the affinities of PCP derivatives modified on the aromatic ring were most strongly influenced by substitutions at the meta position.[15,16] In general, electron-withdrawing groups decreased affinity while electron-donating groups increased the affinity for the PCP binding site. In addition, substitution of the phenyl by a 2-thienyl

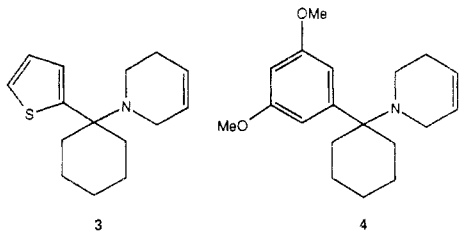


Figure 1. Chemical structures of 1-[1-(2-thienyl)cyclohexyl]-1,2,3,6-tetrahydropyridine (**3**) and 1-[1-(3,5-dimethoxyphenyl)-cyclohexyl]-1,2,3,6-tetrahydropyridine (**4**).

Scheme I. Preparation of 1-(1-Phenylcyclohexyl)-1,2,3,6-tetrahydropyridines

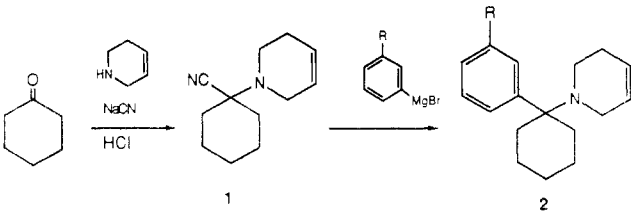


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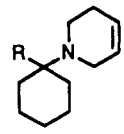
Table II



compd no.	R	K_i for displacement of [^{3}H]TCP,[a] nM	formula	yield, %	mp, °C	anal.
3	2-thienyl	12	$C_{15}H_{21}NS$·HCl	37	173–174	C,H,N
4	3,5-dimethoxyphenyl	8600	$C_{19}H_{27}NO_2$·HCl	26	201–203	C,H,N

[a] Note: The experimentally determined K_i values for PCP and TCP were found to be 80 and 12 nM, respectively.

group, as in 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), was found to greatly increase affinity.

On the basis of these findings we sought to examine compounds which combined those affinity enhancing modifications of the aryl and piperidine portions of the PCP structure into one molecule. In this study we report the in vitro and in vivo PCP-like properties and potential neuroprotective effects of some 1-[1-arylcyclohexyl]-1,2,3,6-tetrahydropyridines.

Chemistry

All compounds were prepared by a modification of the two-step procedure of Maddox et al.,[17] wherein an appropriately substituted aryl Grignard reagent is reacted with a 1-(1-cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (1) to provide the 1-(1-arylcyclohexyl)-1,2,3,6-tetrahydropyridines (2) (Scheme I). Compound **2d** was prepared from the reaction of 1 and the Grignard reagent prepared from 1-(3-bromophenoxy)-1-ethoxyethane (5), followed by acid hydrolysis. Compound **2g** was prepared by acidic hydrolysis of dioxolane **2e**. The oxime **2h** was prepared from the reaction of **2g** and hydroxylamine hydrochloride in the presence of sodium acetate.

Results and Discussion

The results obtained from the [^{3}H]TCP binding experiments are, with a few notable exceptions, in agreement with the proposal that electron-withdrawing groups at the meta position of PCP reduce affinity for the PCP binding site while electron-donating groups increase affinity. Thus, compounds **2b**, **2d**, and **2j** had higher affinity than the parent compound (**2a**), while the aldehyde (**2g**), oxime (**2h**), fluoro (**21**), and trifluoromethyl (**2n**) compounds showed lower affinity (Table I). Compound 4 (Figure 1, Table II) was prepared on the supposition that electron-donating groups at both meta positions might further enhance the binding affinity, but this proved not to be the case. The thienyl compound 3 (Figure 1, Table 2) shows improved affinity over **2a**. The low affinities of **2c** and **2e** indicate that large groups are not well tolerated at this position. A 3-methyl substituent, although slightly electron donating, apparently was ineffective in promoting binding, possibly due to its lack of a heteroatom. This result would tend to indicate that, although the electronic configuration at this position is of importance, some other electronic interaction with the binding site is likely to be involved. This is exemplified by the improvement of binding in compounds **2b** and **2d** over the parent compound **2a**. One discrepancy to this hypothesis can be seen in the methylthio compound **2i**. Although the methylthio group is both electron donating and possesses a heteroatom, the PCP binding site affinity is greatly reduced relative to the parent compound.

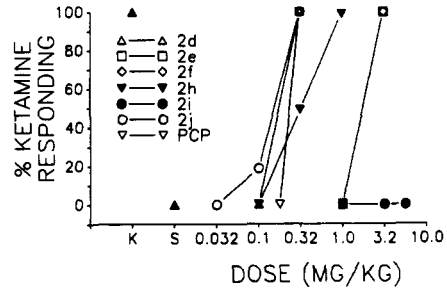


Figure 2. Discriminative ketamine-like responding of selected drugs in rhesus monkeys.

Table III. Protection against Hypobaric-Ischemic Neuronal Degeneration

compd no.	dose, mg/Kg	outcome
2a	2	complete protection
2b	2	no protection
2d	2	toxic—no protection in survivors
2j	2	toxic to all animals
	1	toxic—no protection in survivors
2m	2	protected 1 of 4 animals
3	2	variable but substantial protection
MK-801	1	complete protection

Compounds were chosen for testing in a drug discrimination paradigm on the basis of their having either high PCP binding site affinity (**2d**, **2j**), or having meta groups previously unreported in the PCP series (**2e**, **2f**, **2h**, **2i**). The results of the discriminative testing are shown in Figure 2. On the whole, the in vivo effects correlate well with the in vitro binding data, with low affinity compounds producing ketamine appropriate responding only at higher doses. A notable exception is the methylthio compound **2i** which was not discriminated as being ketamine-like even at higher doses. The relatively low oxidation potential of sulfur might suggest that the compound is rapidly metabolized to a behaviorally inactive sulfoxide or sulfone derivative.

Compounds **2a**, **2b**, **2d**, **2j**, **2m**, and **3** were examined in a baby rat hypoxia/ischemia model in order to estimate their potential as neuroprotective agents. The results of this study (Table III) do not support the notion that compounds with higher affinity also display the best neuroprotective effects. Compounds **2d** and **2j**, which have binding affinities similar to that of MK-801, were toxic to the animals and also produced no neuronal protection. While compounds **2b** and **2m** were less toxic, these compounds similarly displayed no significant amount of protection. The 3,4-dehydro derivatives of PCP (**2a**) and TCP (**3**) showed the best neuroprotective characteristics, with **2a** being protective in all animals.

In summary, a series of 1-(1-phenylcyclohexyl)-1,2,3,6-tetrahydropyridine derivatives were prepared and exam-

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ined. The results indicate that these PCP analogues display in vitro and in vivo pharmacological properties which are in accord with known structure–activity relationships in the PCP series. The neuroprotective characteristics of this series, however, cannot be predicted solely by the use of PCP binding site binding affinities as a guide to neuroprotective effectiveness.

Experimental Section

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR were recorded on a Varian XL-300 instrument, and mass spectra were obtained on a Finnegan 1015D instrument. All spectral data were consistent with the assigned structures. Where elemental analysis are indicated only by symbols for the elements, results obtained were within 0.4% of the theoretical values. Unless otherwise indicated, hydrochloride salts were prepared by dissolving the free base in ethyl acetate followed by the addition of a solution of gaseous HCl in ethyl acetate. Methanesulphonate (MsOH) salts were prepared in ethyl acetate.

Animals used in the drug discrimination studies were maintained in accordance with the University of Michigan Committee on the Use and Care of Animals and guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1985). Animal protocols for the ischemia studies were approved by the Committee on the Humane Care of Laboratory Animals at Washington University Medical School.

Male rats (140–160 g) were obtained from Taconic Farms Inc. Adult male rhesus monkeys (8.4–10.0 kg) used in this study had been previously trained to descriminate reliably between ketamine (Ketalar, Warner-Lambert/Parke-Davis) and saline. The monkeys had, on previous occasions, received many different drugs including other PCP-like compounds. Other than the training dose of ketamine, the monkeys used in this study had not received other drugs for at least one week prior to the current study.

Drug doses for all studies were calculated in mg/kg of body weight.

1-(1-Cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (1). Hydrochloric acid solution (97 mL of 37%, 1.16 mol) was slowly added to slurry of 1,2,3,6-tetrahydropyridine (96.3 g, 1.16 mol) and 500 mL of ice. To this were added sodium cyanide (59.9 g, 1.05 equiv), cyclohexanone (114.2 g, 1.16 mol), and water (500 mL). The reaction was stirred overnight. The reaction mixture was then extracted with methylene chloride (2 × 500 mL), and the extracts were dried ($MgSO_4$) and concentrated to give the oily amino nitrile (260.6 g, 97%): IR (neat) 2214 cm^{-1}; ^{1}H NMR ($CDCl_3$) 5.8 (m, 1 H), 5.7 (m, 1 H), 3.18 (m, 2 H), 2.75 (t, 5.6 Hz, 2 H), 2.15–2.2 (m, 4 H), 1.3–1.85 (m, 8 H).

1-(3-Bromophenoxy)-1-ethoxyethane (5). A solution of 3-bromophenol (66.2 g, 0.382 mol) and ethyl vinyl ether (132 mL, 3.6 equiv) in ethyl acetate (260 mL) was acidified with 5 mL of ethyl acetate saturated with HCl gas. The reaction was allowed to stand overnight after which it was washed quickly with saturated sodium bicarbonate solution (50 mL), dried by filtration through a pad of magnesium sulfate, and concentrated to give the protected bromophenol product as an oil (89 g, 95%).

Preparation of Meta-Substituted 1-(1-Phenylcyclohexyl)-1,2,3,6-tetrahydropyridines. Representative Procedures. 1-[1-(3-Methoxyphenyl)cyclohexyl]-1,2,3,6-tetrahydropyridine (2b). 1-(1-Cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (3.2 g, 16.84 mmol) was dissolved in 10 mL of dry tetrahydrofuran, and the resulting solution was added dropwise to a solution of (3-methoxyphenyl)magnesium bromide prepared by stirring 9.45 g of 3-bromoanisole (3 equiv) in 50 mL of dry THF with 15 g of magnesium turnings or 4 h. After the addition was complete the reaction was allowed to stand for 10 h. The mixture was then poured into a separatory funnel containing 100 mL of water and 50 mL of ether. The aqueous layer was discarded. The remaining organic mixture was washed with 20 mL of 1 N HCl solution. The ethereal layer was then discarded. The acidic aqueous layer was basified with use of 30 mL of 1 N NH_4OH solution and extracted with ether (50 mL × 2). The extracts were dried (Na_2SO_4), concentrated, and converted to the hydrochloride salt. After one recrystallization from ethyl acetate (50 mL), 3.4 g of **2b**·HCl was isolated by filtration (39%): mp 209–211 °C; ^{1}H NMR (CD_3OD) 7.50 (t, J = 8 Hz, 1 H), 7.24 (dd, J = 8, 1 Hz, 1 H), 7.20 (d, J = 1 Hz, 1 H), 7.10 (m, 1 H), 5.89 (m, 1 H), 5.68 (m, 1 H), 3.86 (s, 3 H), 3.8 (m, 2 H), 3.45 (m, 2 H), 2.9–3.1 (m, 4 H), 1.7–2.6 (m, 8 H). Anal. C, H, N.

1-[1-(3-Hydroxyphenyl)cyclohexyl]-1,2,3,6-tetrahydropyridine (2d). 1-(3-Bromophenoxy)-1-ethoxyethane (9.8 g) was vigorously stirred with 15 g of magnesium turnings in 50 mL of dry tetrahydrofuran for 6 h to ensure complete formation of the Grignard reagent. A solution of 1-(1-cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (1, 1.9 g, 10 mmol) in 2 mL of dry THF was then added in one portion, and the resulting solution was stirred overnight. The reaction was decanted into a separatory funnel containing 20 mL of water and 20 mL of ether. The aqueous layer was discarded. The ethereal layer was then washed with 20 mL of 2.0 N HCl solution. The organic layer was discarded. After 20 min, the aqueous was basified with 8 mL of concentrated NH_4OH and extracted with methylene chloride (4 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated to give 21 g of the crystalline free base (82%). The hydrochloride salt was prepared in 2-propanol: mp 226–228 °C; ^{1}H NMR (CD_3OD) 7.40 (t, J = 8 Hz, 1 H), 7.15 (dd, J = 8, 1 Hz, 1 H), 7.10 (d, J = 1 Hz, 1 H), 6.96 (dd, J = 8, 1 Hz, 1 H), 5.91 (bd, J = 8 Hz, 1 H), 5.69 (bd, J = 8, Hz, 1 H), 3.8–3.9 (m, 2 H), 3.4–3.5 (m, 2 H) 2.9–3.1 (m, 4 H), 1.7–2.6 (m, 8 H). Anal. C, H, N.

1-[1-(3-Acetoxyphenyl)cyclohexyl]-1,2,3,6-tetrahydropyridine (2j). Acetyl chloride (0.37 mL, 1.1 equiv) was added dropwise at room temperature to a stirred solution of phenol **2d** (1.2 g, 1.0 equiv) in dry THF (50 mL) and triethylamine (1.42 g, 3 equiv). The copious white precipitate was filtered, and the filtrate was evaporated to provide an oil. Treatment of the oil with gaseous HCl in ethyl acetate and recrystallization from ethyl acetate afforded the acetate ester hydrochloride (1.35 g, 97%): mp 215–217 °C; ^{1}H NMR (CD_3OD) 7.65 (m, 2 H), 7.51 (s, 1 H), 7.32 (d, J = 7 Hz, 1 H), 5.9 (m, 1 H), 5.69 (bd, J = 8 Hz, 1 H), 3.8–3.9 (m, 2 H), 3.4–3.5 (m, 2 H), 2.9–3.1 (m, 4 H), 2.35 (s, 3 H), 1.7–2.6 (m, 8 H). Anal. C, H, N.

1-[1-(3-Carbonylphenyl)cyclohexyl]-1,2,3,6-tetrahydropyridine (2g). A solution of dioxolane **2e** (3.5 g, 11.2 mmol) in 20 mL of 1.5 N HCl solution was warmed to 40 °C for 10 h. After the solution was cooled to room temperature, concentrated NH_4OH (3 mL) was added, and the mixture was extracted with methylene chloride (3 × 20 mL). The extracts were dried (Na_2SO_4) and concentrated. The methanesulfonate salt was prepared in and recrystallized from ethyl acetate (3.38 g, 82%): mp 211–213 °C. Anal. C, H, N.

1-[1-[3-(*N*-Hydroxyimino)phenyl]cyclohexyl]-1,2,3,6-tetrahydropyridine (2h). To a solution of **2g** (240 mg, 0.89 mmol) in 95% ethanol (3 mL) was added a solution of hydroxylamine hydrochloride (2 equiv) and sodium acetate (3 equiv) in water (3 mL). The resulting mixture was stirred overnight, extracted with ether, dried, and concentrated to give the base (250 mg, 99%) which was converted to the hydrochloride salt **2h**·HCl: mp 233–236 °C; ^{1}H NMR (CD_3OD) 8.20 (s, 1 H), 7.91 (s, 1 H), 7.77 (d, J = 8 Hz, 1 H), 7.70 (d, J = 8 Hz, 1 H), 7.60 (dd, J = 8, 8 Hz, 1 H), 5.91 (bd, J = 8 Hz, 1 H), 5.67 (bd, J = 8, Hz, 1 H), 3.8–3.9 (m, 2 H), 3.4–3.5 (m, 2 H), 2.9–3.1 (m, 4 H), 1.7–2.6 (m, 8 H). Anal. C, H, N.

Binding Studies. These studies were conducted as previously described by Jacobson et al.[18] using a tissue homogenate preparation of fresh whole rat brain minus cerebellum. Incubation was carried out at 5 °C with [^{3}H]TCP as the radioligand. Rapid filtration was done through filters presoaked in 0.03% polylysine. The inhibition constant (K_i) for determination of the affinity of the compound for the PCP binding site was calculated with the Cheng–Prusoff[19] equation with use of our predetermined Kd for TCP (16.5 nM) from Scatchard analysis. TCP (10 μM) was used

(18) Jacobson, A. E.; Harrison, E. A., Jr.; Mattson, M. V.; Rafferty, M. G.; Rice, K. C.; Woods, J. H.; Winger, G.; Solomon, R. E.; Lessor, R. A.; Silverton, J. V. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 110.

(19) Cheng, Y. C.; Prusoff, W. H. *Biochem Pharmacol.* **1973**, *22*, 3099.

for the determination of the nonspecific binding. Nonspecific binding was found to be less than 5% of total in each case.

Drug Discrimination Studies. Three adult rhesus monkeys (*Maccaca mulatta*) trained previously to discriminate between sc injections of saline and ketamine were maintained at 90% of their free-feeding weights. During experiments monkeys were seated in Plexiglas chairs located within well ventilated, sound-attenuating chambers that were equipped with two response levers, a food receptacle, and an array of colored stimulus lights. Training sessions consisted of a 10-min time out and a response period during which the stimulus lights were illuminated and ten food pellets were delivered upon completion of 100 consecutive lever presses on the lever designated correct according to the injection during the first minute of the time out (left lever, saline; right lever, ketamine). For some sessions a dose of 1.78 mg/kg of ketamine was administered during the time our of one cycle and for that cycle and a subsequent noninjection cycle only responding on the right lever produced food. The number of saline cycles that preceded the ketamine cycle varied and for some sessions saline was administered for all cycles. Test sessions were identical with training sessions except for food was delivered after 100 consecutive responses on either lever and increasing doses of other drugs were administered during consecutive time out periods. Drugs were considered to have substituted for ketamine if they produced an average of >90% responding on the right lever. All drugs were dissolved in sterile water and administered sc in the back in a volume of 0.1 mL/kg body weight.

Hypobaric-Ischemic Neuronal Degeneration Assay. Ten-day old Sprague-Dawley rats, both male and female (weight 20 g) were subjected to unilateral carotid ligation under halothane anesthesia. During and immediately after surgery the pups were kept on a warming pad that maintained their body temperature at 36° C as measured by a skin surface microprobe. After recovery from anesthesia, each pup was injected subcutaneously with 1–2 mg/kg of an experimental drug in a volume of 100 μL. The dose of 1–2 mg/kg was chosen as it had previously shown that 1 mg/kg MK-801 protects infant rat brains from hypoxia/ischemia.[11] Each drug dose was tested in 4–6 animals. A group of 10–12 injected pups were placed in a hypobaric chamber with a volume of 2370 mL that was immersed in a water bath. The internal temperature of the chamber was maintained at 36 °C throughout the incubation period. Air pressure within the chamber was gradually reduced from 760 to 225 mmHg over a 1-min period, maintained at 225 mm for 75 min and then allowed to return to 760 mm over a 1-mm period. The pups remained in the chamber for another 2 h at which time they were sacrificed by intra-cardiac perfusion under chloral hydrate anesthesia with a phosphate buffered solution of 1% paraformaldehyde and 1.5% glutaraldehyde. Perfused brains were removed, sliced, and additionally fixed with 1% osmium tetroxide, dehydrated in graded ethanols, cleared in toluene, and embedded in Araldite. Sections (1 μm) were cut on a Sorvall MT-2B ultratome, stained with methylene blue/azure II and evaluated by light microscopy.

By employing the protocol described above, several brain regions have been shown to suffer substantial neuronal damage.[20] However, there is some individual variation in response. Since the carotid artery is ligated only on one side, only the ipselateral side of the brain is damaged and the contralateral side serves as a control. Carotid ligation alone for 2 h and 75 min or subjection to the same hypobaric conditions without carotid ligation does not induce brain damage.[20] The degree of damage in the medial hebenula nuclei, the brain region most sensitive to hypobaric/ischemic damage in infant rat, was evaluated by investigators who were blind to treatment regimens. Drugs were tested with the goal of identifying agents which would provide complete protection at the dose at which the standard (MK-801) has been shown to be protective.[11] Complete protection was considered to be no necrotic cells in the medial habenula nucleus ipselateral to the carotid ligation of every animal in a given dose group in which the contralateral nucleus was damaged.

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