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Monohydroxymetabolites of phencyclidine (PCP): activities and urinary excretion by rat, dog and mouse

E. J. CONE*, D. B. VAUPEL, D. YOUSEFNEJAD, *National Institute on Drug Abuse, Addiction Research Center, Lexington, Kentucky, U.S.A.*

Phencyclidine [1-(1-phenylcyclohexyl)piperidine; PCP] is metabolized in man and various animal species by hydroxylation at the C_4 position of the piperidine and cyclohexane rings to yield to 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP) and 4-phenyl-4-piperidinocyclohexanol (PPC) (Lin et al 1975; Wong & Bieman 1976; Cone et al 1980; Martin et al 1980). Other polar metabolites have been detected (Misra et al 1979; Martin et al 1980) or identified (Wong & Bieman 1976). The pharmacological activities of PCHP and PPC have been assumed to be negligible (James & Scholl 1976; Burns & Lerner 1978; Bailey et al 1978; Sioris & Krenzelok 1978; Bailey & Guba 1980) based on the work of McCarthy & Potter (1963), who reported only stimulant effects for these compounds in the monkey. Recently, significant biological activities for PCHP and PPC have been reported in the rat and dog (Domino 1978; Vaupel & Cone 1981).

We have evaluated the rotarod activity and the LD50 values of PCHP and PPC in the mouse and their excretion profiles in mouse, rat and dog as a means of determining the contributions made by these metabolites to the pharmacological activity of the parent compound.

Materials and methods

PCP, PCHP, PPC and 1-(1-phenylcyclohexyl)morpholine (internal standard) were obtained from Research Technology Branch, Division of Research, National Institute on Drug Abuse (Rockville, Maryland, USA). Their purity was checked by t.l.c., g.c. and mass spectrometry. heptafluorobutyric anhydride (HFBA) was purchased from Pierce Chemical Co., Rockford, Ill., USA. All other chemicals were of reagent grade quality.

Urinary excretion. One female and one male beagle dog (10·4 and 12·2 kg), six male rats (albino Wistar, 270–340 g) and 28 male mice (Swiss-Webster Cox, 28–40 g) were housed in metal cages equipped with stainless steel urine collection funnels. A single dose of PCP.HCl was administered intraperitoneally to dogs (0·5 mg kg^{-1}), rats (10 mg kg^{-1}) and mice (5 mg kg^{-1}) and urine was collected once each 24 h for 3 days, pooled for each species and frozen until analysis.

Analysis of urine samples. A sample (5–10 ml) of each urine was mixed with IS (10 μg) and extracted with hexane at pH 10·0 as previously reported by Cone et al (1981). The samples were extracted either untreated or after acid hydrolysis with 10% (v/v) hydrochloric acid (115 °C and 1·27 kg cm^{-2}). Extracts were evaporated to dryness under dry nitrogen and derivatized with HFBA (20 μl) in benzene (0·1 ml) at 85–90 °C for 60 min, cooled, and then ammonium hydroxide solution (1 ml of 5% v/v) added and the sample shaken and centrifuged. Benzene (50 μl) was added and the aqueous layer discarded; 1–3 μl of the organic phase was analysed.

G.c.–m.s. analysis was on a Finnigan Model 4021 in the chemical ionization mode. For g.c. a glass column (2 mm, i.d. 1·84 m) packed with 3% SE-30 on Gas-Chrom Q (100/120 mesh), was coupled to the mass spectrometer by a glass-lined stainless steel tube. Injector, column, interface oven and ion source temperatures were maintained at 165, 190, 250 and 250 °C, respectively. Carrier and reagent gases were methane or helium. The electron energy was 70 eV and the multiplier voltage 1·35 KV. Total ion scans of extracted standards and samples were collected over 80–600 AMU.

A Perkin-Elmer Sigma 2 gas chromatograph was used with a nitrogen sensitive detector for g.l.c. analysis. The column was as above. The conditions were: bead voltage, 450 mV; carrier flow (helium), 20 ml min^{-1}; air, hydrogen flow adjusted for maximum response; injector, 160 °C; detector, 250 °C; column, 180 °C. Daily standard curves were constructed for PCP, PCHP and PPC using the peak height ratio method. The curves were linear over the concentrations tested (0·1–0·8 μg ml^{-1} PCP, 0·05–1·6 μg ml^{-1} PCHP, PPC) with correlation coefficients (r) ≥ 0·98. The lower limits of the assay were ca 0·01 μg ml^{-1} for PCP and 0·05 g ml^{-1} for PCHP and PPC. Recoveries of PCP, PCHP and PPC from untreated or acid-hydrolysed samples ranged from 65–75%.

Rotarod and acute toxicity bioassays. Adult, male SIM $(SW)_f$ BR mice were given PCP.HCl in 0·9% NaCl and the free base forms of the isomers, PCHP and PPC, in a 3:2 solution of 8·5% lactic acid and 1 M sodium hydroxide. Drugs were administered i.p. at 0·01 ml g^{-1} weight.

The rotarod method as described by Su et al (1980) was used, each mouse being sequentially tested for 120 s at 6 min intervals for 1 h. Relative potency estimates were determined by parallel line bioassay analysis and ED50 (μmol kg^{-1}) values were calculated using regression analysis of individual curves.

Acute lethal doses were determined in drug naive mice over 24 h. The 4 h LD50 values (μmol kg^{-1}) and relative toxicity estimates were determined according to Litchfield & Wilcoxon (1949). LD50 values were also combined with rotarod data to compute the therapeutic index (LD50/ED50) for PCP and its metabolites.

* Correspondence.

Results

In vivo activities of PCHP and PPC in the mouse. The 4-hydroxypiperidine metabolite, PCHP, was some 5 times more active than the 4-hydroxycyclohexyl metabolite, PPC, in the mouse rotarod assay but both were less active than the parent, PCP. The ED50's (95% confidence limits) for PCHP and PPC were 63·1 (57·1–69·9) and 328·3 (305·0–353·4), respectively, compared with 17·6 (16·4–18·9) for PCP. Their relative potencies were: PCP, 1·00; PCHP, 0·27 (0·17–0·36); PPC, 0·052 (0·02–0·094).

The 4 h LD50 (95% confidence limit) for PCHP was 434 (419–450), for PPC 635 (607–664), and for PCP 280 (263–298) mg kg^{-1}. Relative toxicities were: PCP, 1·00; PCHP, 0·65 (0·58–0·71); PPC, 0·44 (0·40–0·49).

Although the metabolites were less potent than PCP, therapeutic indices were PCP 16, PCHP 7 and PPC 2.

Metabolite identification. The presence of both PPC and PCHP in varying amounts in the urine of the mouse, rat and dog, was confirmed (g.c.–m.s.) with authentic standards.

A new hydroxymetabolite was detected in the urine of the mouse and dog (Rt 6·10 min; relative Rt = 1·22). Its mass spectrum (helium CI) was qualitatively similar to that of PPC with key ions at *m/z* 455, 412, 378, 242, 91 and 84. The most abundant ion was observed at *m/z* 242 (loss of HFBA group) whereas the most abundant ion for PPC was at *m/z* 200. Key ions for both compounds were observed at *m/z* 378 (loss of phenyl group), *m/z* 200 (loss of HFBA-propyl) and *m/z* 157 (loss of HFBA and piperidine). From these data, a tentative structural assignment of hydroxylation at C2 or C3 of the cycloalkyl ring of PCP was made.

Measurement of PCP, PCHP and PPC in urine. After a single intraperitoneal dose of PCP.HCl (Table 1), overall recoveries of PCP and metabolites from untreated urine were low for all species (ca 2%). Acid-hydrolysis increased recoveries for the mouse (8·3%) and dog (11·5%), but there was only a slight increase for the rat (3·0%). Most of the drug accounted for was excreted in 0–24 h by all species. However, the dog excreted substantial amounts of drug and metabolites during 24–28 h. Both PCP and PPC were detectable throughout the 72 h collection periods but PCHP was often not detectable after 24–48 h.

The amounts of PPC and PCHP in urine varied with the species, time of collection and sample treatment. Only small amounts of PCHP (0·1–0·3%) were found for any species in contrast to PPC, the total excretion of which ranged from 0·3% for the rat to 6·9% for mouse and 9·0% for dog. The degree of conjugation of PPC (e.g., difference between untreated and acid-hydrolysed urines) also varied with species. Rat urine contained the least amount of conjugated PPC (ca 50%) followed by mouse (82%) and dog (98%).

The unidentified hydroxymetabolite was estimated to represent about 2·0% of the administered dose for the mouse and 0·5% for the dog. Substantial portions of the administered dose were left unaccounted for in all species.

Table 1. Mean urinary excretion of phencyclidine (PCP) and monohydroxy-metabolites. Results are mean of triplicate analyses expressed as percent administered dose ± standard error.

Species (N) time (h)	Sample treatment[a]	PCP	PPC	PCHP	Total
Mouse (30)					
0–24	UT	1·23 ± 0·21	1·23 ± 0·28	0·05 ± 0·04	2·51
	HYD	1·27 ± 0·24	6·60 ± 1·30	0·05 ± 0·01	7·92
24–28	UT	0·04 ± 0·02	0	0	0·04
	HYD	0·03	0·25 ± 0·01	0	0·28
48–72	UT	0·02	0	0	0·02
	HYD	0·02 ± 0·01	0·09 ± 0·01	0	0·11
Rat (7)					
0–24	UT	1·60 ± 0·06	0·15	0·17 ± 0·01	1·92
	HYD	1·83 ± 0·07	0·19 ± 0·01	0·14 ± 0·01	2·16
24–28	UT	0·31 ± 0·01	0	0	0·31
	HYD	0·31 ± 0·01	0·10 ± 0·06	0·01 ± 0·01	0·42
48–72	UT	0·47 ± 0·05	0	0	0·47
	HYD	0·45 ± 0·05	0	0	0·45
Dog (2)					
0–24	UT	0·89 ± 0·11	0·10 ± 0·02	0·06	1·05
	HYD	1·16 ± 0·14	6·27 ± 0·73	0·24 ± 0·03	7·67
24–48	UT	0·65 ± 0·10	0·05 ± 0·02	0·05	0·75
	HYD	0·86 ± 0·07	2·23 ± 0·51	0·09 ± 0·01	3·18
48–72	UT	0·17 ± 0·04	0	0	0·17
	HYD	0·10 ± 0·01	0·54 ± 0·07	0·03	0·67

[a] UT = untreated samples; HYD = acid-hydrolysed samples.

Discussion

No evidence was obtained for hydroxylation of PCP on the aromatic ring by the species examined but a new monohydroxymetabolite with the probable site of hydroxylation occurring at C2 or C3 of the cycloalkyl ring was detected in the mouse and dog.

Overall recoveries of unchanged PCP from urine of all species following a single dose of PCP were low (1–2%), consistent with other reports of detection of substantial amounts of polar unidentified metabolites (Misra et al 1979; Martin et al 1980). The amounts of hydroxylated metabolites varied with species. The rat produced least (ca 0·5%), whereas mouse and dog produced substantially more (ca 8%). Stereoselectivity of hydroxylation (ratio of PPC to PCHP) also varied with species. Rat produced almost equal amounts of PPC and PCHP, whereas mouse and dog produced much larger amounts of PPC than PCHP. A similar species trend was noted in the degree of conjugation of the hydroxymetabolites. Rat excreted little hydrolysable material, but mouse and dog urines contained sizeable amounts.

Both PCHP and PPC displayed less activity than PCP in the mouse rotarod and LD50 studies. Although the rotarod assay is generally considered to be non-specific, a high correlation has been obtained for rotarod potency estimates of PCP derivatives and those of discriminative stimulus studies in the rat (Su et al 1980). Considering the lower potency estimates of the metabolites and their amounts in urine, they are unlikely to contribute substantially to the profile of effects produced by PCP, but resulting therapeutic index values revealed relatively lower margins of safety for the metabolites compared with PCP, hence they may have a greater potential for contributing to a toxic PCP reaction if high amounts of the parent drug have been taken.

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Histamine tachyphylaxis in canine isolated airways: role of endogenous prostaglandins

C. BRINK, P. G. DUNCAN, J. S. DOUGLAS*, *John B. Pierce Foundation Laboratory, 290 Congress Avenue, New Haven, CT 06519, U.S.A.*

The basal tone of respiratory smooth muscle in vitro is related to the endogenous production of small amounts of prostaglandins (Coburn et al 1974; Orehek et al 1975; Duncan et al 1980). Agonist contraction or mechanical stimulation enhanced release of these mediators (Orehek et al 1973; Gryglewski et al 1976). On the basis of these data it has been proposed that the locally released prostaglandins are important in the maintenance of resting tone and antagonize the response of respiratory smooth muscle during contraction.

Recently Anderson et al (1979) proposed another hypothesis for the role of prostaglandins in airway muscle namely, receptor desensitization. These authors reported that canine isolated tracheal muscle when contracted with histamine became refractory and this tachyphylaxis could be reversed by exposure to the anti-inflammatory agent indomethacin. While many reports have related increased prostaglandin production to the desensitization of β-adrenoceptors (Gryglewski & Ocetkiewicz 1974; Douglas et al 1977) few have suggested their role as mediators in histamine receptor desensitization (Krzanowski et al 1980). We have determined whether histamine was tachyphylactic in canine bronchi and if the tachyphylaxis was associated with altered prostaglandin synthesis.

Methods

Animals and tissues. Male mongrel dogs 13–23 kg, were anaesthetized with intravenous sodium pentobarbitone (30 mg kg^{-1} i.v.). The chest cavity was opened and a lung lobe was removed and immersed in cold Tyrode solution of composition mM: NaCl, 139·2; KCl, 2·7; $CaCl_2$, 1·8; $MgCl_2$, 0·49; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4 and glucose, 5·5; pH 7·4.

Bronchi from the 5th generation were dissected out, spirally cut and equilibrated in Tyrode solution in 10 ml organ baths under an initial load of 5 g. In four experiments, lung parenchymal strips were also cut from the lung lobes and placed in similar tissue baths under a 1 g load. All preparations were allowed to equilibrate for 90 min in Tyrode solution at 37 °C gassed with 5% CO_2 in O_2. Force was measured isometrically with Statham strain gauges (Model UC3) and was displayed on Honeywell two-channel pen recorders (Electronik 19). After each experiment the tissues were dried (65 °C) for 12 h and weighed. The change in basal tone of each preparation was determined from the records by calculating the resting tone (g mg^{-1} tissue dry weight) at the beginning and end of the equilibration period (equilibration ratio) as well as before and after an incubation with indomethacin (incubation ratio).

Concentration-effect curves to acetylcholine. Concentration-effect curves were produced by adding acetylcholine (1 to 200 μM) in a volume <0·5 ml, in random order, to the tissue bath. When the response to the agonist reached a plateau, the bath fluid was exchanged for fresh Tyrode solution and the preparation was allowed to return passively to its resting tone. Contractions produced by

* Correspondence.