C=O) cm^{-1}. Anal. ($C_{32}H_{37}N_9O_6 \cdot 0.5H_2O$) C, H, N.

$Ba(OH)_2 \cdot 8H_2O$ (126 mg, 0.4 mmol) was added to a stirred solution of the diester **7** (129 mg, 0.2 mmol) in 50% EtOH (10 mL). After 60 h, a solution of Na_2SO_4 (57 mg, 0.4 mmol) in H_2O was added, and the mixture was stirred vigorously for 5 min. The precipitate of $BaSO_4$ was removed by filtration and the filtrate was concentrated to a small volume by rotary evaporation and then freeze-dried. The product was desalted by passage through a DEAE-cellulose column, which was eluted first with a large volume of H_2O and then with 6% NH_4HCO_3. Appropriately pooled fractions of the latter eluent were freeze-dried to give a yellow solid (98 mg, 82% yield); R_f 0.80 (cellulose, pH 7.4 phosphate buffer); IR (KBr) ν 3340, 1615 cm^{-1}. Anal. ($C_{24}H_{29}N_9O_6 \cdot 3H_2O$) C, H, N.

Note Added in Proof: After this manuscript was submitted for publication, a symposium monograph appeared in which it was discussed that tetrahydrofolate and folate, i.e., 4-oxo, compounds analogous to **3** were excellent substrates for hog liver FPGS.[27] It was also stated in that symposium that compound **2** was not a substrate for rat liver enzyme.[28] Hence, the results shown in Figure 1 for mouse liver FPGS may be of more general applicability to FPGS from other mammalian sources.

Acknowledgment. We thank Drs. Peter Danenberg and John Holcenberg for their critical reviews of this manuscript and Patrice Johnston-Turner for her help in preparing it. This work was supported by Public Health Service Grants CA 27605 (R.G.M.), CA 25394, and CA 30897 (to A.R.) from the National Institues of Health, DHHS.

Registry No. **2**, 68352-96-5; **3**, 91425-22-8; **4**, 91425-23-9; **5**, 19741-14-1; **6**, 89106-05-8; **7**, 91425-24-0; MeAPA-Glu, 59-05-2; MeAPA-Glu-Glu, 41600-13-9; FPGS, 63363-84-8; 4-aminobutyric acid, 56-12-2; *N,O*-bis(trimethylsilyl)-4-aminobutyric acid, 39538-11-9; *N,O,O*-tris(trimethylsilyl)-L-glutamic acid, 15985-07-6; methyl 4-aminobutyrate hydrochloride, 13031-60-2; glutamic acid, 56-86-0.

(27) Bognar, A. L.; Cichowicz, D. J.; Shane, B. In "Chemistry and Biology of Pteridines"; Blair, J. A., Ed.; DeGruyter and Co.: New York, 1983; p 627.

(28) McGuire, J. J.; Hsieh, P.; Bertino, J. R.; Coward, J. K. In "Chemistry and Biology of Pteridines"; Blair, J. A., Ed.; DeGruyter and Co.: New York, 1983; p 615.

N-Allyl Analogues of Phencyclidine: Chemical Synthesis and Pharmacological Properties

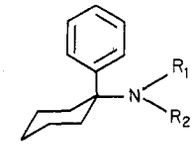
Asher Kalir,[†,§] Shoshana Teomy,[†] Adina Amir,[†] P. Fuchs,[†] Sung A. Lee,[‡] Elzbieta J. Holsztynska,[‡] Wieslaw Rocki,[‡] and Edward F. Domino*[,‡]

Israel Institute for Biological Research, Ness Ziona, Israel, and Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109. *Received April 18, 1983*

Several *N*-allyl derivatives of 1-phenylcyclohexylamine (PCA) were prepared, and their pharmacology was briefly characterized. The mono- and diallyl derivatives **4–7** had phencyclidine-like activities in mice but were less potent behaviorally than phencyclidine (PCP). None were PCP antagonists. In vitro these compounds were competitive inhibitors of butyrylcholinesterase (BChE) and protected against inhibition by DFP. In addition, these agents displaced tritiated *N*-methyl-4-piperidyl benzilate from mouse-brain homogenates and inhibited the effects of acetylcholine on isolated guinea pig ileum. None of these in vitro effects correlated with their PCP-like behavioral activity in vivo in mice.

N-(1-Phenylcyclohexyl)piperidine (phencyclidine, PCP, Sernyl, angel dust, peace pill) was originally developed as an analgesic anesthetic for humans but later withdrawn from clinical use because of its undesirable mental side effects. In recent years, it has become a drug of widespread abuse in the United States, resulting in severe incidents of psychoses including delirium, hallucinations, depression, coma, seizures, etc.

Although extensive pharmacological studies of PCP have been done, neither its mechanism of action is known nor has a specific pharmacological antagonist of PCP been found. Since it is known that the introduction of an allyl group into a pharmacologically active centrally acting compound may cause either an antagonism (e.g., naloxone) or potentiation/addition (e.g., barbiturates) of the original effects, we prepared and studied the pharmacology of a number of *N*-allyl derivatives (**4–7**) of 1-phenylcyclohexylamine (**1**, PCA) that are related to PCP. *N*-Allylnormetazocine and particularly the (+) enantiomer have been reported to produce PCP-like responses in laboratory animals.[1,2]



1, $R_1 = R_2 = H$
2, $R_1 = Me$; $R_2 = H$
3, $R_1 = Et$; $R_2 = H$
4, $R_1 = H$; $R_2 = CH_2{=}CHCH_2$
5, $R_1 = Me$; $R_2 = CH_2{=}CHCH_2$
6, $R_1 = Et$; $R_2 = CH_2{=}CHCH_2$
7, $R_1 = R_2 = CH_2{=}CHCH_2$

Results

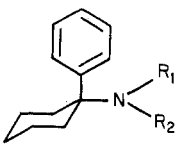
Chemistry. The synthesis of the *N*-allyl derivatives was carried out according to Scheme I. (Alkylamino)cyclohexanecarbonitriles (**11**) were prepared from cyclohexanone (**8**), alkylamine hydrochloride (**9**), and sodium cyanide (**10**). Their reaction with phenyllithium yielded *N*-alkyl-1-phenylcyclohexylamines (**2**, **3**),[3,4] which with allyl

[†] Israel Institute for Biological Research.
[‡] University of Michigan.
[§] Present address: Department of Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.
* Address reprint requests.

(1) Brady, K. T.; Balster, R. L.; May, E. L. *Science* **1982**, *215*, 178.
(2) Shannon, H. E. *Eur. J. Pharmacol.* **1982**, *84*, 225.

0022-2623/84/1827-1267$01.50/0

Table I. Protection of BChE against DFP by Phencyclidine and *N*-Allyl-1-phenylcyclohexylamines[a]



compd	R_1	R_2	concn, M	$t_{1/2}$, min	f[b]	$C_{(f=2)}$,[c] M	K_i, M	K_D, M
PCP			0	2.1	1			
		$-(CH_2)_5-$	4×10^{-7}	2.6	1.26	1.2×10^{-6}	5.2×10^{-7}	9.1×10^{-6}
			8×10^{-7}	3.6	1.73			
			4×10^{-6}	9.2	4.48			
			0	2.3	1.00			
			4×10^{-6}	2.2	0.95			
3	C_2H_5	H	8×10^{-6}	5.8	2.52	1.7×10^{-5}	5.8×10^{-5}	
			4×10^{-5}	6.0	2.60			
			0	2.4	1.00			
			2×10^{-6}	3.0	1.22			
4	H	$CH_2CH=CH_2$	3×10^{-6}	4.2	1.71	4.9×10^{-6}	2.7×10^{-5}	7.0×10^{-5}
			4×10^{-6}	6.4	2.61			
			8×10^{-6}	5.8	2.35			
			0	1.9	1.00			
			4×10^{-6}	4.1	2.15			
5	CH_3	$CH_2CH=CH_2$	8×10^{-6}	4.2	2.21	5.6×10^{-6}	5.6×10^{-6}	1.5×10^{-5}
			4×10^{-5}	14.8	7.76			
			0	2.3	1.00			
			4×10^{-7}	3.0	1.33			
6	C_2H_5	$CH_2CH=CH_2$	8×10^{-7}	3.9	1.71	9.7×10^{-7}	2.6×10^{-6}	1.4×10^{-5}
			4×10^{-6}	11.5	5.11			
			0	1.9	1.00			
			4×10^{-7}	2.3	1.18			
7	$CH_2CH=CH_2$	$CH_2CH=CH_2$	9×10^{-7}	4.1	2.12	8.5×10^{-7}	5.0×10^{-7}	2.5×10^{-5}
			4×10^{-6}	11.4	5.89			

[a] The data are expressed in K_i values and their affinities for the muscarinic receptor as K_D values. [b] $f = t_{1/2}$(protection)/$t_{1/2}$(control). [c] C is the concentration at which $t_{1/2}$ was increased by twofold.

bromide (**12**) gave the desired allyl compounds **5** and **6**. An alternative route of synthesis consisted in allylation of **11** to tertiary amino nitriles **13** and **14** that with phenylmagnesium bromide produced **5** and **6**. The *N*-monoallyl- and *N,N*-diallyl-1-phenylcyclohexylamine (**4** and **7**, respectively) were prepared by allylation of 1-phenylcyclohexylamine (**1**).[5,6]

Pharmacology. Butyrylcholinesterase Inhibition and Protection against DFP. PCP and its derivatives are reversible cholinesterase inhibitors.[7] Maayani et al.[7] reported that PCP and its derivatives protected both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) against irreversible inactivation by the organophosphate DFP.[8,9]

We have conducted a similar investigation on the inactivation of BChE by PCP and its *N*-allyl analogues **4–7**. In addition, the degree of protection by PCP and its *N*-allyl derivatives **4–7** against the inactivation of BChE by DFP have been investigated. BChE activity was determined by using acetylthiocholine as the substrate as described in the Experimental Section. The K_m of BChE for acetylthiocholine was 5×10^{-4} M. All of the PCP derivatives tested (**3–7**) were found to act as the reversible competitive inhibitors of BChE. The degree of protection was measured by comparing the half-life ($t_{1/2}$) observed in the time course of inhibition of BChE by DFP in the presence and absence of PCP and its *N*-allyl derivatives. DFP was used as an irreversible inhibitor of BChE in a concentration of 2.5×10^{-8} M. Enzyme activity as a function of time ranged between 20% and 70%. The k_2 value was calculated from the equation given in the Experimental Section and found to be 1.45×10^{-7} M/min. This result is consistent with that of Jandorf et al.[10]

The degrees of protection of BChE activity against DFP by PCP and its *N*-allyl analogues **4–7** are summarized in Table I. In order to compare the potencies of the *N*-allyl derivatives as protective agents, the value $C_{(f=2)}$ was calculated. $C_{(f=2)}$ is the concentration at which the half-life ($t_{1/2}$) in the time course of inhibition of BChE by DFP increased by twofold in the presence of PCP and its *N*-allyl analogues. It may be seen in Table I that the *N*-allyl derivatives **4–7** protected BChE against irreversible inactivation by DFP. The degree of protection against a constant concentration of DFP varied with the concentration of the analogue used. Previous studies with PCP[7] and other reversible cholinesterase inhibitors[9] have suggested that both the degree of anticholinesterase activity and the ability of each of the compounds to compete with DFP for a specific active site in the cholinesterase molecule contribute to the protective property of these compounds. The affinities of the *N*-allyl derivatives for BChE and their protective effects were increased with enlargement of the cationic head at the nitrogen atom of the *N*-allyl derivatives. Compounds **3** and **4** possess a secondary nitrogen

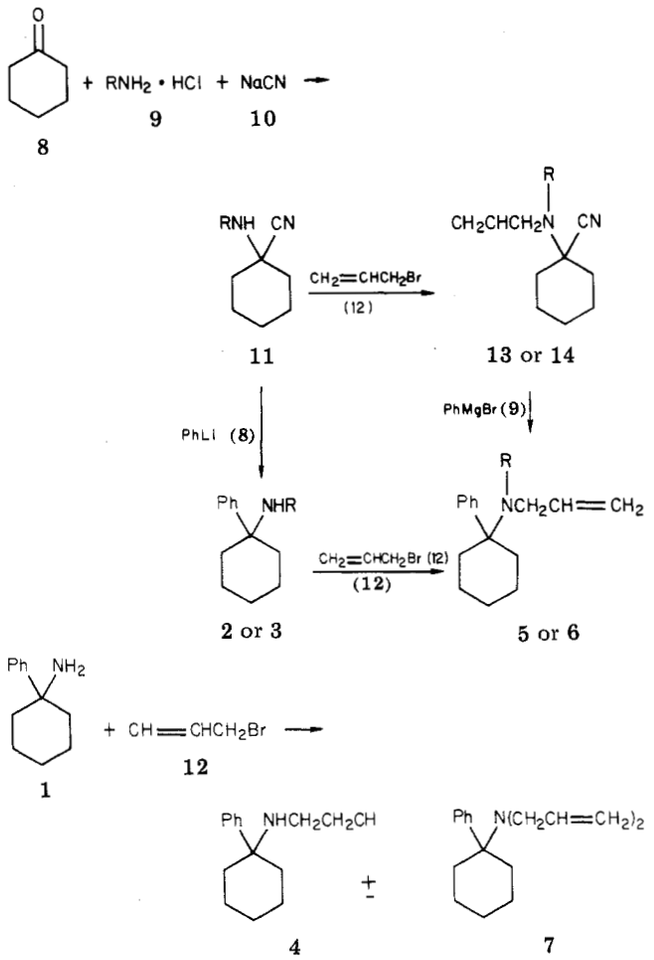
(3) Kalir, A.; Edery, H.; Pelah, Z.; Balderman, D.; Porath, G. *J. Med. Chem.* **1969**, *12*, 473.
(4) Maddox, H.; Godefroi, E. F.; Parcell, R. F. *J. Med. Chem.* **1965**, *8*, 230.
(5) Kalir, A.; Pelah, Z. *Isr. J. Chem.* **1967**, *5*, 223.
(6) Balderman, D.; Kalir, A. *Synthesis* **1978**, 24.
(7) Maayani, S.; Weinstein, H.; Ben-Zvi, N.; Cohen, S.; Sokolovsky, M. *Biochem. Pharmacol.* **1974**, *23*, 1263.
(8) Koelle, G. B. *J. Pharmacol. Exp. Ther.* **1946**, *88*, 232.
(9) Koelle, G. B. "Cholinesterase and Anticholinesterase Agents"; Springer-Verlag: West Berlin–Gottingen–Heidelberg, 1963; p 336.
(10) Jandorf, B. J.; Michel, H. O.; Schaffer, N. K.; Egan, R.; Summerson, W. H. *Discuss. Faraday Soc.* **1955**, *20*, 134.

Table II. Relative Potency in Mouse Platform Performance and Acute Toxicity of Phencyclidine and *N*-Allyl Analogues

drug	ED_{50}, μmol/kg	95% confidence limits	relative potency	LD_{50}, μmol/kg	95% confidence limits	acute toxicity (rel to PCP)
PCP	9.60	7.63–12.1	1.00	258	242–275	1.00
N-allyl-PCP (4)	20.4	14.8–28.1	0.55	344	314–377	0.75
N-allyl-*N*-methyl-PCA (5)	27.2	19.3–38.3	0.41	516	480–555	0.50
N-allyl-*N*-ethyl-PCA (6)	22.6	19.3–26.4	0.42	441	362–538	0.59
N,*N*-diallyl-PCA (7)	35.6	30.6–41.1	0.27	ND[a]	ND	ND

[a] Not determined.

Scheme I



atom, while **5**, **6**, **7**, and PCP possess a tertiary nitrogen atom. It seems that a tertiary nitrogen atom contributes to greater protective potency compared to a secondary nitrogen atom. Thus, PCP, **6**, and **7** are more effective than **4** and **5**. The differences noted could also be due to the various degree of lipophilicity of these compounds as pointed out by a referee. From an analysis of the data in Table I, the following SAR data would be generated:

compd	obsd $-\log K_i$	calcd $-\log K_i$	π
PCP	6.28	6.39	2.50
3	4.24	4.36	1.00
4	4.57	4.50	1.10
5	5.26	5.17	1.60
6	5.59	5.85	2.10
7	6.30	5.98	2.20

$$-\log K_i = (1.35 \pm 0.17) + (3.01 \pm 0.23)$$

$$r = 0.9715,\ F = 67.1,\ n = 6$$

Since the difference between the observed and predicted values were within experimental error of the biological measurement, this model would seem adequate.

Table III. Interaction between Phencyclidine and the *N*-Allyl Derivatives[a]

compd	dose, μmol/kg	ratio of failed/ tested	% failing the test
	Experiment 1[b]		
PCP	13.2	2/6	33.3
N-allyl-PCA ED_{10}	10.0	6/6	100
+ PCP	13.2		
N-allyl-PCA ED_{20}	11.5	5/6	83.3
+ PCP	13.2		
N,*N*-diallyl-PCA ED_{10}	26.0	6/6	100
+ PCP	13.2		
N,*N*-diallyl-PCA ED_{20}	29.0	6/6	100
+ PCP	13.2		
N-allyl-*N*-methyl-PCA ED_{10}	16.0	6/6	100
+ PCP	13.2		
N-allyl-*N*-methyl-PCA ED_{20}	19.0	6/6	100
+ PCP	13.2		
	Experiment 2[b]		
PCP	9.60	11/24	45.8
N-allyl-*N*-ethyl-PCA ED_{10}	12.4	10/12	83.3
+ PCP	9.60		
N-allyl-*N*-ethyl-PCA ED_{20}	15.2	10/12	83.3
+ PCP	9.60		

[a] The dose of PCP close or equal to its ED_{50} was administered ip 5 min after ip injection of an *N*-allyl analogue at a dose equal to its ED_{10} or ED_{20}. [b] Experiments 1 and 2 were performed at different times.

Muscarinic Receptor Binding. Maayani et al.[7] suggested that the psychotomimetic activities of PCP and its derivatives were due to their anticholinergic effects via a direct interaction with the muscarinic cholinergic receptor. The affinities of three of the *N*-allyl analogues of PCP for the muscarinic receptor have been investigated by using the methods described in the Experimental Section. The affinities of PCP and its *N*-allyl analogues **4**–**7** were expressed in terms of dissociation constants (K_D) of the complexes of the muscarinic receptor and the *N*-allyl derivatives. The results are shown in Table I. Of the three compounds tested (PCP, **4**, and **5**), PCP was the most potent and **4** the least.

Behavioral Activity and Toxicity. The relative potencies of PCP and the *N*-allyl derivatives **4**–**7** were investigated by using the mouse platform test of Coughenour et al.[11] This test measures the degree of motor incoordination in untrained mice. In addition, the interaction between PCP and its *N*-allyl analogues has been investigated for the possible role of the *N*-allyl derivatives as pharmacological antagonists of PCP.

Table II shows the ED_{50} values, relative potency at peak effect, and LD_{50} values as determined by the Litchfield and Wilcoxon method.[12] All of the *N*-allyl derivatives (**4**–**7**)

(11) Coughenour, L. L.; McLean, J. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
(12) Litchfield, J. T.; Wilcoxon, F. *J. Pharm. Exp. Ther.* **1949**, *96*, 99

tested showed weaker potencies than PCP. The order of potency at peak effect was PCP > *N*-allyl-PCA (**4**) ≥ *N*-allyl-*N*-ethyl-PCA (**6**) ≥ *N*-allyl-*N*-methyl-PCA (**5**) > *N*,*N*-diallyl-PCA (**7**). The order of acute toxicity (LD_{50}) was PCP > *N*-allyl-PCA (**4**) > *N*-allyl-*N*-ethyl-PCA (**6**) ≥ *N*-allyl-*N*-methyl-PCA (**5**). Table III shows the interaction between phencyclidine and its *N*-allyl analogues. In all cases, coadministration of PCP in doses equal or close to its ED_{50} and *N*-allyl analogues **4**–**7** (in ED_{10} and ED_{20} doses) resulted in a slight synergistic effect on mouse platform performance. The administration of the *N*-allyl-*N*-ethyl derivative **6** in ED_{10} and ED_{20} doses, combined with a lower dose of PCP (ED_{25}), also showed synergistic action (not shown in Table III). A referee has suggested that the "synergistic effect observed may be ascribed to protein binding. Thus, the allyl derivative would compete with PCP for its binding site with serum proteins, then more of the latter would be available in the free form which is also available to the brain". PCP binds to human plasma proteins about 78%. The binding is linear up to 1000 ng/mL.[17] In our studies plasma concentrations of PCP reached 100 ng/mL. The relationship proposed by Goldstein et al.[18] between bound fraction and drug concentration, *X*, predicts that the lower is *X*, the larger is the bound fraction. If one assumes that a low enough concentration of PCP the bound fraction is 90%, then the free fraction is 10%. If binding is now reduced by a competing drug to 85%, then free fraction is 15%, i.e., a 50% increase in free fraction available to the brain.

Conclusions

From the above results, one can conclude the following.

1. *N*-Allyl analogues of PCP (**4**–**7**) show pharmacological effects similar to those of PCP itself.

2. The substitution of a piperidine ring of PCP by mono- or diallylamine causes an alteration both in the affinities for the BChE and the muscarinic receptor and in the potency of behavioral activity in mice. There is no correlation between in vitro activity and PCP-like behavioral effects.

3. The *N*-allyl analogues of PCP (**4**–**7**) do not show a partial agonistic/antagonistic effect of PCP, although their potencies of PCP-like behavioral effects were weaker than PCP, but when administered concurrently show slight synergistic action.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian FT-80 instrument and were consistent with the assigned structures. The hydrochlorides were dissolved in $CDCl_3$ with Me_4Si as the reference.

1-(Methylamino)cyclohexanecarbonitrile (11, R = Me). A solution of 30% of aqueous methylamine (approximately 230 mL) was cautiously added to 170 mL (2 mol) of concentrated hydrochloric acid and 300 g of crushed ice, and then the mixture was stirred with 196 g (2 mol) of cyclohexanone and 105 g (2 mol) of 98% sodium cyanide. After the mixture stood overnight, the organic layer was separated, dried over magnesium sulfate, and distilled to give 170 g (62%) of the nitrile **11** (R = CH_3), bp 110 °C (15 mm).

1-(Methylallylamino)-1-cyclohexanecarbonitrile (13). A mixture of 13.8 g (0.1 mol) of the above nitrile, 25 g (0.12 mol) of allyl bromide (**12**), and 15 g of anhydrous potassium carbonate in 100 mL of acetonitrile was stirred and refluxed for 4 h. The solid inorganic material was filtered off, and the filtrate was concentrated, diluted with benzene, washed with water, dried, and distilled to give 31.5 g (59%) of **13**, mp 115–130 °C (18 mm). An analytical sample was redistilled at 125 °C (20 mm). Anal. ($C_{11}H_{18}N_2$) C, H, N.

***N*-Allyl-*N*-methyl-1-phenylcyclohexylamine (5, R = Me).** A solution of 30 g of nitrile **13** in 100 mL of benzene was added to phenylmagnesium bromide (from 42 g of bromobenzene and 6.8 g of magnesium in 150 mL of ether). The reaction mixture was stirred and refluxed for 2 h and decomposed, and the basic material was distilled to give 5 g of **5**, bp 105–120 °C (1 mm), identical with the compound described below.

***N*-Methyl-1-phenylcyclohexylamine (2).** A solution of 34.5 g (0.25 mol) of **11** (R = Me) in 100 mL of benzene was added to phenyllithium (from 118 g of bromobenzene and 13.5 g of lithium in 400 mL of ether). The mixture was heated for 30 min and decomposed, and the basic material was isolated and distilled to give 25 g (62%) of the known **2**.

***N*-Allyl-*N*-methyl-1-phenylcyclohexylamine (5).** A mixture of 15 g (0.08 mol) of **2**, 15 g of anhydrous potassium carbonate, and 15 g (0.12 mol) of **12** in 150 mL of acetonitrile was stirred and refluxed for 6 h. The solid was filtered off and the filtrate concentrated, dissolved in ether, filtered again, and treated with ethereal hydrogen chloride. The solid (13 g, 61%) was recrystallized from acetone to give 5 g (24%) of pure hydrochloride of **5**: mp 178–179 °C; NMR δ 7.50 (s, 5 H), 7.20 (s, 1 H), 6.19 (m, 1 H), 5.17 (m, 2 H), 4.03 (d, 2 H), 2.48 (d, 3 H). Anal. ($C_{16}H_{24}ClN$) C, H, Cl, N.

***N*-Allyl-*N*-ethyl-1-phenylcyclohexylamine (6).** The hydrochloride salt, mp 163–164 °C, was analogously obtained in 55% yield from *N*-ethyl-1-phenylcyclohexylamine: NMR δ 7.50 (d, 5 H), 7.20 (s, 1 H), 6.12 (m, 1 H), 5.18 (m, 2 H), 4.0 (m, 2 H), 3.30 (m, 2 H), 1.35 (t, 3 H). Anal. ($C_{17}H_{16}ClN$) C, H, Cl, N.

***N*-Allyl-1-phenylcyclohexylamine (4).** A mixture of 8.8 g (0.05 mol) of 1-phenylcyclohexylamine (**1**),[6] 7.0 g of anhydrous potassium carbonate, and 7.0 g of allyl bromide in 70 mL of ethanol and 25 mL of dimethylformamide was stirred and heated at 50 °C for 3 h. The mixture was cooled, filtered, and concentrated and the basic material distilled to give 9.3 g (86%) of **4**, bp 115–120 °C (1.5 mm).[4] The compound was converted to the HCl salt, mp 209–211 °C (ethanol–ethyl acetate).[4] Anal. ($C_{15}H_{22}ClN$) C, H, Cl, N.

***N*,*N*-Diallyl-1-phenylcyclohexylamine (7).** A mixture of 6.5 g (0.037 mol) of **1**, 10 g of **12**, and 12 g of potassium carbonate in 75 mL of acetonitrile was stirred and heated for 3 h. The mixture was cooled, filtered, and concentrated and the residue dissolved in ether, filtered again, and converted to the hydrochloride with ether–HCl. The crude hydrochloride (7 g, 65%) was recrystallized from ether–methanol and then from THF–methanol to give 3.1 g (29%) of crystals, mp 135–137 °C. Anal. ($C_{18}H_{26}ClN$) C, H, Cl, N.

Pharmacology. Determination of BChE Activity. BChE activity was determined by the method of Ellman et al.[13] BChE was obtained from horse serum (specific activity, 3 units/mg). Acetylthiocholine (1.87×10^{-3} M) was used as the substrate with 0.025 enzyme unit in 0.1 M phosphate buffer (pH 7.4) at 25 °C. The development of absorbancy vs. time was recorded.

Rates of Inactivation of BChE by DFP. DFP was obtained from the Aldrich Chemical Co. DFP (2.5×10^{-8} M) was added to 1.0 mL (0.5 unit) of BChE. Aliquots of 50 μL were assayed for residual enzyme activity after various incubation times at 25 °C by diluting to 3.0 mL in the cuvettes. The increase in absorbancy at 412 mm with time was observed spectrophotometrically. This technique yielded a pseudo-first-order constant from which the second-order rate constant was calculated.

The kinetic constants (second-order rate constant, k_2) were calculated from the equation that is valid for pseudo-first-order reaction conditions:[9] $k_2[I]t = \ln A_0/A_t$, where A_0 = enzyme activity at time 0, A_t = enzyme activity after t minutes of incubation with inhibitor, [I] = concentration of inhibitor, k_2 = second-order rate

(13) Ellman, G. L.; Cortney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
(14) Kloog, Y.; Sokolovsky, M. *Brain Res.* **1978**, *144*, 31.
(15) Augustinson, K. S. *Biochem. Biophys. Acta* **1966**, *128*, 351.
(16) Miller, L. C.; Tainter, M. L. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261, 17.
(17) Giles, H. G.; Corrigall, W. A.; Khouw, V.; Seilers, E. M. *Clin. Pharmacol. Ther.* **1982**, *31*, 77.
(18) Goldstein, A.; Aronow, L.; Kalman, S. M. "Principles of Drug Action"; Harper & Row: New York, 1968; p 140.

constant, and t = incubation time in minutes.

Degree of Protection. Various concentrations of the *N*-allyl derivatives were added to 1.0 mL (0.5 unit) of BChE. After incubation for 3 min, 50 μL aliquots were assayed for enzyme activity. DFP (2.5×10^{-8} M) was added, and 50 μL aliquots were assayed for residual enzyme activity after various incubation times.

Affinity for the Muscarinic Receptor. Dissociation constants (K_D) of the complexes of the muscarinic receptor and the *N*-allyl derivatives were measured by two different procedures:

(a) Competition experiments used the S_1 supernatant fraction of mouse-brain homogenate.[14] Tritiated *N*-methyl-4-piperidyl benzilate ([^{3}H]-4NMPB) was incubated with the fraction of mouse-brain homogenate with and without the test compound in various concentrations. Nonspecific binding of the labeled ligand was determined,[14] and the difference in binding and the K_D value was calculated.

(b) Acetylcholine was added in a concentration at which 90% of the maximum contraction of the isolated guinea pig ileum was produced.[15] The compounds were tested as inhibitors of this contraction and dose–response curves were derived for calculation of K_D values from the dose ratios. The results by both methods were similar.

Mouse Platform Test. Male albino HAP ICR Swiss mice, 25–30 g, obtained from Harlan Sprague–Dawley Industries, Indianapolis, IN, were used for these experiments. The animals were housed in the rodent facility with a day–night cycle of 0700–1900 light and 1900–0700 dark and a room temperature of 21–25 °C. Food and water were supplied ad lib. and removed for the 3–4-h period the experiment. Experiments were performed between 0800 and 1200. The mouse platform was built to the specifications of Coughenour et al.[11] All mice were screened prior to use for passing the platform test. At 5, 15, 30, 60, 90, and 120 min after drug injection, the animals were placed, one mouse each, on a horizontal platform. The platforms were rotated 180° within 10 s and the mice scored on the basis of falling off the screen, failure to reach the top, and reaching the top of the screen during the period of 60 s. Gross behavior and the condition of the mice were observed within 3–4 h after injection. All compounds were administered ip with use of 0.9% NaCl as a vehicle in the form of a solution or a very fine suspension. In the case of a solubility problem of compound **7**, the suspensions were homogenized mechanically for 2 min with a glass Potter-Evelhejm homogenizer immediately prior to injection. Drug dosage was expressed in micromoles/kilogram. The volume of the dosing solution was 0.01 mL/g of body weight. The effect of the vehicle was checked in separate experiments. Four to six different doses of the same drug were tested daily. Six animals were used for each dose level. The experiments were replicated two to four additional times on different days. The quantal dose–response curves were determined graphically with use of all of the combined data obtained at the time of the peak effect. As described earlier, ED_{50} as well as 95% confidence limits were estimated from probit analysis using the Litchfield and Wilcoxon method.[12] For accurate dose–effect curves, 0% and/or 100% effects were corrected according to the method of Miller and Tainter[16] and Litchfield and Wilcoxon.[12]

Acute Toxicity Test. Compounds **4–6** were administered ip to groups of six male HAP ICR Swiss mice weighing 25–35 g. The LD_{50} values were estimated from the 1-day survivals of mice subjected to the behavioral screen. Deaths were determined 24-h postinjection. All quantal data were evaluated by the same method as the ED_{50}.

Interaction between PCP and the *N*-Allyl Derivatives. Male HAP ICR Swiss mice weighing 20–30 g were used under the same conditions as previously described. *N*-Allyl derivatives of PCA (**4–7**) were administered ip to groups of six mice at ED_{10} and ED_{20}. Five minutes later, PCP was administered ip at the dose close to ED_{50}. Then the same mouse platform tests were carried out as previously described.

Acknowledgment. This work was supported by Grant No. 1638 from the U.S.–Israel Binational Foundation and by NIDA Grant DA-01531 for which we express our appreciation.

Registry No. **1**, 2201-24-3; **2**, 2201-16-3; **4**, 2201-49-2; **4**·HCl, 2185-95-7; **5**, 91281-23-1; **5**·HCl, 91281-24-2; **6**, 82845-39-4; **6**·HCl, 91281-25-3; **7**, 91281-26-4; **7**·HCl, 91281-27-5; 11, 6289-40-3; **12**, 106-95-6; **13**, 91281-28-6; cyclohexanone, 108-94-1; methylamine hydrochloride, 593-51-1; phenyl bromide, 108-86-1; phenyllithium, 591-51-5; *N*-ethyl-1-phenylcyclohexylamine, 2201-15-2; butyrylcholinesterase, 9001-08-5; **2b**, 91281-35-5; **3b**, 91281-29-7; **3c**, 91281-32-2; **3d**, 91281-36-6; **4a**, 2014-75-7; **4a**·HCl, 34946-13-9; **4b**, 91281-31-1; **4b**·HCl, 91281-30-0; **4c**, 91281-34-4; **4c**·HCl, 91281-33-3; **4d**, 91281-38-8; **4d**·HCl, 91281-37-7; 2-methyl-2-oxazoline, 1120-64-5; 4-mercaptophenol, 637-89-8; ethyl iminoacetate hydrochloride, 2208-07-3; (±)-2-amino-1-propanol, 6168-72-5; dopamine β-hydroxylase, 9013-38-1; monamine oxidase, 9001-66-5.

Conformationally Restrained Fentanyl Analogues. 2. Synthesis and Analgetic Evaluation of Perhydro-1,6-naphthyridin-2-ones[1a]

Ronald F. Borne,*[†] E. Kim Fifer,[†,1b] and I. W. Waters[‡]

Departments of Medicinal Chemistry and Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi 38677. *Received December 1, 1983*

Conformational flexibility of the *N*-acyl portion of fentanyl-type analgetics was restricted through the synthesis of novel perhydro-1,6-naphthyridin-2-one derivatives. Neither the cis-fused derivative (**5a**), the trans-fused derivative (**5b**), nor the enamide **8a** possessed analgetic activity in the mouse tail-flick assay, reaffirming the sensitivity of this portion of 4-anilidopiperidine analgetics to conformational restraint.

Fentanyl (**1a**) is the most potent analgetic currently available (Sublimaze) in this country and has been the subject of a number of structural and conformational studies. Substitution of either the ethyl[2,3] or the phenyl[4] moiety on the propananilido group generally decreases activity, although an *o*-methoxy substituent appears to slightly increase opiate receptor binding.[5] A sharp reduction in binding affinity results from substitution of carbon for the amide nitrogen.[6] Although the *cis*-(+)-3-methyl analogue **1b** is reported to be 6684 times as potent as morphine,[7] a methyl group in the 2-position or 2,5-di-

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmacology.

(1) (a) For paper 1, see: Borne, R. F.; Law, S. J.; Kapeghian, J. C.; Masten, L. W. *J. Pharm. Sci.* **1980**, *69*, 1104. (b) Abstracted in part from the Ph.D. dissertation of E.K.F.
(2) Janssen, P. A. J.; Van der Eycken, C. A. M. In "Drugs Affecting the Central Nervous System"; Burger, A., Ed.; Marcel Dekker: New York, 1968; Vol. 2, Chapter 2.
(3) Maryanoff, B. E.; Simon, E. J.; Gionnini, T.; Gorissen, H. *J. Med. Chem.* **1982**, *25*, 913.
(4) Grossman, S.; Moser, U.; Mutschler, E. *Arch. Pharm. (Weinheim, Ger.)* **1978**, *311*, 1010.

0022-2623/84/1827-1271$01.50/0