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ACKNOWLEDGMENTS AND ADDRESSES

Received September 4, 1970, from the **Merck Sharp & Dohme Research Laboratories, West Point, PA 19486*, and the †*Graduate Hospital, School of Medicine, University of Pennsylvania, Philadelphia, PA 19486*

Accepted for publication December 14, 1970.

The authors acknowledge the contributions of Mr. H. Wallick with the microbiological assays, of Mr. M. Grell with the computer simulations, and of Dr. J. L. Ciminera and Dr. N. R. Bohidar with the statistical analyses. Special thanks are extended to Dr. T. J. Macek for his encouragement and support.

‡ Present address: Squibb Institute for Medical Research, New Brunswick, NJ 08903

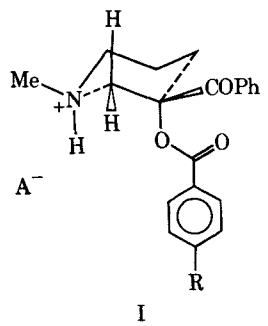
Stereochemical Investigations of Local Anesthetic Action

M. LOKHANDWALA*, D. B. PATEL, H. PATEL†, P. C. MERKER, A. SHAFI'EE‡, and G. HITE§

Abstract □ The toxicities and local anesthetic activities in a variety of assay systems are reported for a series of 1-alkyl-3-benzoyl-3-acyloxypiperidines. In solution, the procaine analog, XI, is conformationally more homogeneous than procaine about the 4-atom aminoethoxy unit and is 8 times more active and 16 times more toxic than procaine on a molar basis. This suggests that the preferred *gauche*-conformation of XI has positive biological significance for local anesthetic receptors on one hand and for CNS and/or cardiac receptors on the other hand, although this stereochemical feature discriminates in favor of CNS and/or cardiac receptors.

Keyphrases □ Anesthetics, local—conformation–activity relationship □ Stereochemical aspects—local anesthetic activity □ 1-Alkyl-3-benzoyl-3-acyloxypiperidines—synthesis □ Pharmacological screening—1-alkyl-3-benzoyl-3-acyloxypiperidines □ IR spectrophotometry—structure □ Polarimetry—conformation

The antipodal and diastereoisomeric potency and toxicity ratios of amino ester local anesthetics vary with the pharmacological tests used in their evaluation, but they never exceed 8 : 1 (1–5). Recent studies (6–9) on the synthesis and local anesthetic activity of asymmetric 1-methyl-3-benzoyl-3-chloropiperidine and 1-methyl-3-benzoyl-3-acyloxypiperidines (I) prompted a more rigorous pharmacological evaluation and comparison of this prototype with the chemically analogous but conformationally less restricted drug, procaine (II), in order to explore the potential relevance of conformational



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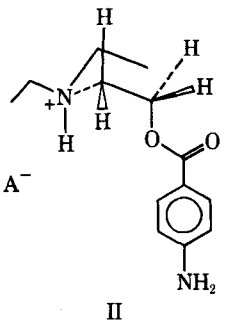

II

restraints (*cf.*, 10, 11) about the 4-atom aminoethoxy unit to potency and/or selectivity. While this investigation is restricted to ethanolamine esters, it is of interest that conformational effects have been studied in the propanolamine series (12).

EXPERIMENTAL[1]

Chemistry

All melting points were obtained in a Hershberg-type (13) silicone (550-Dow)-filled melting-point apparatus equipped with Anschutz full-immersion thermometers and are uncorrected. The samples were placed in the silicone bath 10° below the reported melting points and heated at a rate of 1–2°/min.

Specific rotations were determined with a Zeiss 0.01° polarimeter in a modified (14) 2-dm., 2-ml. syringe-filling polarimeter tube.

IR spectra were run with a Perkin-Elmer 421 double-grating spectrophotometer as mulls in mineral oil between NaCl plates. Assignment of absorption bands, believed accurate to within ±5 $cm.^{-1}$, were made by analogy with reported values (15).

Petroleum ether refers to the fraction boiling from 30–60°. Solutions of free amines in apolar solvents were clarified with activated charcoal[2] and dried simultaneously with anhydrous sodium sulfate; they were then filtered through sintered glass. Solvents were evaporated in a water bath under reduced pressure. All base washings were performed with saturated aqueous $NaHCO_3$. All acid washings were performed with 3 *N* HCl. Unless otherwise stated, the HCl salts were prepared in Et_2O using HCl gas, dried, and recrystallized.

(+)-1-Methyl-3-benzoyl-3-benzoyloxypiperidine (III) Hydrochloride—To 13.0 g. (59.3 mmoles) of IV, (+)-1-methyl-3-benzoyl-3-hydroxypiperidine, m.p. 72.5–73.0°, $[\alpha]_D^{25}$ (absolute EtOH) +11.4 ± 0.3° (c 5.00) [lit. (10, 11) 72.5–73.0°, +11.4°] in 100 ml. of pyridine was added 18 g. (80 mmoles) of benzoic anhydride. The solution was allowed to reflux for 24 hr. After evaporation of the solvent, the residue was mixed with 100 ml. of 3 *N* HCl. The mixture was washed with $HCCl_3$, made basic with Na_2CO_3, and extracted with $HCCl_3$. The extract was dried, filtered, and evaporated to give an oil which, on clarification in and crystallization from petroleum ether, afforded 9.28 g. (28.7 mmoles, 48%) of III: m.p. 65–66°; $[\alpha]_D^{28}$ (absolute EtOH) +61.4 ± 0.4° (c 9.00); IR 1705 $cm.^{-1}$ (C=O, aromatic ester), 1676 $cm.^{-1}$ (C=O, aromatic ketone), no OH band in the 3500-$cm.^{-1}$ region.

Anal.—Calcd. for $C_{20}H_{21}NO_3$: C, 74.3; H, 6.55; N, 4.33. Found: C, 74.0; H, 6.62; N, 4.14.

The HCl salt was crystallized from 2-PrOH[3]: m.p. 234–235°.

[1] Elemental analyses were performed by Weiler and Strauss, Oxford, England.
[2] American Norit Co.
[3] 2-PrOH = isopropyl alcohol.

Anal.—Calcd. for $C_{20}H_{22}ClNO_3$: C, 66.8; H, 6.16; Cl, 9.85; N, 3.89. Found: C, 66.8; H, 6.16; Cl, 9.71; N, 3.92.

(±)-1-Methyl-3-benzoyl-3-benzoyloxypiperidine (V) Hydrochloride—To 4.35 g. (18.7 mmoles) of VI, (±)-2-methoxy-2-phenyl-5-methyl-1-ox-5-azaspiro[2.5]octane, b.p. 115–119°/2 mm. [lit. (7, 11, 16) b.p. 70–71°/0.08 mm., *cf.*, 8, b.p. 117–119°/2 mm.], consisting of a mixture of both diastereoisomers (16–19) in 50 ml. of dry Et_2O, was added 5 g. (41 mmoles) of benzoic acid in 50 ml. of dry Et_2O. After 24 hr. at room temperature, the solvent was evaporated and replaced with $HCCl_3$. The mixture was washed with base and then with H_2O, dried, filtered, and evaporated. The residue was clarified in and crystallized from petroleum ether to give 3.9 g. (12.2 mmoles, 65%) of V: m.p. 114–115° [lit. (8) m.p. 114–115°]; IR 1708, 1675 cm.$^{-1}$. The HCl salt, m.p. 201–202° [lit. (8) m.p. 201–202°], was crystallized from 2-PrOH.

(±)-1-Methyl-3-benzoyl-3-(*o*-chlorobenzoyloxy)piperidine (VII) Hydrochloride—Treatment of 6.5 g. (41.5 mmoles) of *o*-Cl-benzoic acid in 300 ml. of Et_2O and 4.35 g. of VI, as in the preparation of V, afforded VII from Et_2O: m.p. 120–122°; IR 1732, 1686 cm.$^{-1}$; and subsequently 6.0 g. (15.3 mmoles, 82%) of the HCl salt from 2-PrOH: m.p. 221–223°: IR 1732, 1679 cm.$^{-1}$.

Anal.—Calcd. for $C_{20}H_{21}Cl_2NO_3$: C, 60.9; H, 5.37; Cl, 17.98; N, 3.55. Found: C, 60.7; H, 5.09; Cl, 17.71; N, 3.39.

(±)-1-Methyl-3-benzoyl-3-(*o*-bromobenzoyloxy)piperidine (VIII) Hydrochloride—Treatment of 8.5 g. (42.3 mmoles) of *o*-Br-benzoic acid and 4.35 g. of VI, as in the preparation of V, afforded VIII from Et_2O; m.p. 149–151°; IR 1743, 1679 cm.$^{-1}$; and subsequently 7.0 g. (15.9 mmoles, 85%) of the HCl salt from 2-PrOH: m.p. 216–218°; IR 1736, 1683 cm.$^{-1}$.

Anal.—Calcd. for $C_{20}H_{21}BrClNO_3$: C, 54.8; H, 4.83; Br, 18.21; Cl, 8.08; N, 3.19. Found: C, 55.1; H, 5.05; Br, 18.00; Cl, 8.00; N, 3.22.

(±)-1-Methyl-3-benzoyl-3-(*p*-hydroxybenzoyloxy)piperidine (IX) Hydrochloride—Treatment of 13.8 g. (0.1 mole) of *p*-OH-benzoic acid in 500 ml. of boiling Et_2O and 7.0 g. (30 mmoles) of VI, as in the preparation of V, afforded IX which was dissolved in 250 ml. of Me_2CO and treated with 5 ml. of 12 *N* HCl. The salt crystallized on cooling and was recrystallized from EtOH to give 8.1 g. (21 mmoles, 70%) of IX·HCl·$^1/_2H_2O$; m.p. 226–227° dec.

Anal.—Calcd. for $C_{20}H_{22}ClNO_4 \cdot {}^1/_2H_2O$: C, 62.4; H, 6.02; Cl, 9.21; N, 3.64. Found: C, 62.8; H, 6.09; Cl, 9.30; N, 3.71.

(±)-1-Methyl-3-benzoyl-3-(*p*-methoxybenzoyloxy)piperidine (X) Hydrochloride—Treatment of 15.2 g. (0.1 mole) of *p*-OCH_3-benzoic acid in 100 ml. of boiling tetrahydrofuran and 7.0 g. of VI, as in the preparation of V, afforded 8.9 g. (25.2 mmoles, 84%) of X. The HCl salt·H_2O was crystallized from EtOH–Et_2O, m.p. 196–198°.

Anal.—Calcd. for $C_{21}H_{24}ClNO_4 \cdot H_2O$: C, 61.8; H, 6.43; Cl, 8.70; N, 3.43. Found: C, 61.8; H, 6.20; Cl, 9.72; N, 3.46.

(±)-1-Methyl-3-benzoyl-(*p*-aminobenzoyloxy)piperidine (XI) Hydrochloride—Treatment of 6.0 g. (44 mmoles) of *p*-NH_2-benzoic acid in 75 ml. of boiling tetrahydrofuran and 4.35 g. of VI, as in the preparation of V, afforded 5.8 g. (17.2 mmoles, 92%) of XI from *n*-hexane–Et_2O, m.p. 145–147°.

Anal.—Calcd. for $C_{20}H_{22}N_2O_3$: C, 71.0; H, 6.55; N, 8.28. Found: C, 71.3; H, 6.35; N, 7.88.

The HCl salt was prepared by adding 17.2 meq. of standardized 2-PrOH–HCl to 5.8 g. of XI in 2-PrOH–tetrahydrofuran (60:40), evaporating the solvent, and recrystallizing the residue from 2-PrOH–Et_2O: m.p. 170–172°; IR 3310, 1705 cm.$^{-1}$.

Anal.—Calcd. for $C_{20}H_{23}ClN_2O_3$: C, 64.1; H, 7.47; Cl, 9.46; N, 7.47. Found: C, 64.0; H, 7.40; Cl, 9.21; N, 7.34.

(±)-1-Methyl-3-benzoyl-3-(*o*-chloro-*p*-aminobenzoyloxy)piperidine (XII) Hydrochloride—Treatment of 7.7 g. (45 mmoles) of *o*-Cl-*p*-NH_2-benzoic acid and 4.35 g. of VI, as in the preparation of XI·HCl, afforded 5.9 g. (15.9 mmoles, 85%) of XII and, subsequently, the HCl salt from 2-PrOH–Et_2O: m.p. 203–205°; IR 3340, 1708, 1673 cm.$^{-1}$.

Anal.—Calcd. for $C_{20}H_{22}Cl_2N_2O_3$: C, 58.7; H, 5.42; Cl, 17.32; N, 6.84. Found: C, 58.3; H, 5.72; Cl, 17.00; N, 7.04.

(±)-1-*n*-Propyl-3-benzoyl-3-hydroxypiperidine (XIII) Hydrochloride—Treatment of 3-benzoyl-3-hydroxypiperidine (XIV) obtained from 7.0 g. (18.5 mmoles) of the *p*-toluenesulfonate salt, m.p. 192–193° [lit. (9) m.p. 192–193°], with 2.75 g. of *n*-PrBr in 75 ml. of DMF at 60° for 48 hr., followed by evaporation of DMF, afforded a residue which was dissolved in H_2O. The solution was made basic with Na_2CO_3 and extracted with petroleum ether. The extract was dried, clarified, filtered, and evaporated to give 2.9 g. (11.7 mmoles, 63%) of XIII: IR 3440, 1678 cm.$^{-1}$. The HCl salt was crystallized from 2-PrOH: m.p. 226–228° dec.; IR 1672 cm.$^{-1}$.

Anal.—Calcd. for $C_{15}H_{22}ClNO_2$: C, 63.5; H, 7.81; Cl, 12.49; N, 4.94. Found: C, 63.5; H, 7.80; Cl, 12.52; N, 4.68.

(±)-1-*n*-Propyl-3-benzoyl-3-benzoyloxypiperidine (XV) Hydrochloride—Benzoylation of 2.47 g. (10 mmoles) of XIII, as in the preparation of III·HCl, afforded 2.72 g. (7.0 mmoles, 70%) of XV·HCl from 2-PrOH–Et_2O: m.p. 181–182°; IR 1719, 1680 cm.$^{-1}$; no OH band in 3500 region.

Anal.—Calcd. for $C_{22}H_{26}ClNO_3$: C, 68.1; H, 6.76; Cl, 9.14; N, 3.61. Found: C, 68.0; H, 6.49; Cl, 9.17; N, 3.88.

(±)-1-*iso*-Propyl-3-benzoyl-3-benzoyloxypiperidine (XVI) Hydrochloride—Treatment of 7.0 g. of XIV·*p*-tosylate with 3.14 g. of 2-PrI, as in the preparation of XIII, gave 2.15 g. (8.7 mmoles, 47%) of 1-*iso*-propyl-3-benzoyl-3-hydroxypiperidine (XVII) which was benzoylated as in the preparation of III·HCl to afford 2.86 g. (8.2 mmoles, 94%) of XVI, IR 1735, 1670 cm.$^{-1}$, and, subsequently, 2.53 g. (6.52 mmoles, 75%) of XVI·HCl from 2-PrOH: m.p. 196–197°; IR 1718, 1673 cm.$^{-1}$.

Anal.—Calcd. for $C_{22}H_{26}ClNO_3$: C, 68.1; H, 6.76; Cl, 9.14; N, 3.61. Found: C, 68.2; H, 6.68; Cl, 9.45; N, 3.63.

(±)-1-Methyl-3-benzoyloxy-3-(α-hydroxybenzyl)piperidine (XVIII)—To V, obtained from 1.64 g. (4.55 mmoles) of V·HCl, were added 10 ml. of 90% EtOH and 0.046 g. of $NaBH_4$. After 24 hr., the solvent was evaporated and the residue was boiled with Et_2O. The Et_2O was washed with H_2O, dried, clarified, filtered, and evaporated to give 1.13 g. (3.5 mmoles, 77%) of product (XVIII and V); IR 3460, 1712, 1678 cm.$^{-1}$. The mixture was dissolved in petroleum ether and, on cooling, afforded crystals which were recrystallized from petroleum ether to give 685 mg. (2.1 mmoles, 46%) of XVIII: m.p. 126–127°; IR 3460, 1712 cm.$^{-1}$. The stereochemistry of this apparently pure diastereoisomer is unknown.

Anal.—Calcd. for $C_{20}H_{23}NO_3$: C, 73.8; H, 7.12; N, 4.30. Found: C, 73.6; H, 7.15; N, 4.57.

(±)-1-*iso*-Propyl-3-benzoyloxy-3-(α-hydroxybenzyl)piperidine (XIX) Hydrochloride—Treatment of 0.3 g. of $NaBH_4$ in 100 ml. of 90% EtOH and XVI, obtained from 1.9 g. (4.85 mmoles) of XVI·HCl, as in the preparation of XVIII, afforded 1.2 g. of XIX: IR 3450, 1708 cm.$^{-1}$, slight absorption in 1680 region. The HCl salt, 1.17 g. (3.0 mmoles, 62%), was crystallized from 2-PrOH–Et_2O, m.p. 225–227°. The stereochemistry of this apparently pure diastereoisomer is unknown.

Anal.—Calcd. for $C_{22}H_{28}ClNO_3$: C, 67.8; H, 7.24; Cl, 9.09; N, 3.59. Found: C, 67.5; H, 7.00; Cl, 9.39; N, 3.51.

Biology

Animals and Experimental Conditions—Male albino mice, guinea pigs, and rabbits were used. These were obtained from a commercial breeder and were housed and fed under standard conditions for 1–2 weeks prior to use. Solutions of all test compounds were prepared immediately prior to use with physiological saline or with distilled water for injection.

ED_{50}—Four standard methods were used for the evaluation of local anesthetic activity.

1. Corneal anesthesia in rabbits: the procedure of Luduena and Hoppe (20) was followed, using four rabbits for each concentration of drug.

2. Intracutaneous wheal in guinea pigs: the technique of Bulbring and Wajda (21) was employed, with six tests on three guinea pigs being performed simultaneously.

3. Sciatic nerve block in guinea pigs: the method of Shackell (22) was used with four guinea pigs for each concentration of drug.

4. Mouse tail: the procedure of Bianchi (23) was employed with 10 mice (average weight 30–35 g.) for each concentration. A minimum of five concentrations was used to determine the dose–response curves.

$T_{1/2}$ Values—Using the method of Bianchi (23), a sufficient number of mice were injected with compounds at doses corresponding to the previously determined ED_{50} values. At least 10 responding mice were selected after 15 min. and retested for local anesthesia every 5 min. thereafter until all test animals recovered. The percentage of mice anesthetized was plotted *versus* time, and the line was fitted by means of regression analysis. The time at which 50% of the test animals remained anesthetized was obtained by extrapolation from the graph.

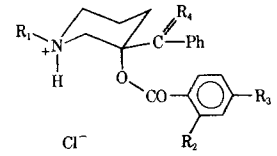

Table I—Local Anesthetic Potency and LD_{50} Values of Some Substituted Piperidines and Control Compounds Procaine, Lidocaine, and Cocaine

Number	Compound R_1	R_2	R_3	R_4	LD_{50}[a], Mouse, Subcutaneous Injection, mg./kg.	ED_{50}[a,b], Mouse Tail, mg./kg.	ED_{50}[a], Guinea Pig Wheal, mg./kg.	ED_{50}[a], Sciatic Nerve, mg./kg.	ED_{50}[d], Rabbit Cornea, %	*TI*[e]	$T_{1/2}$[e–h] at ED_{50}, min.	Sat.[e,h,i] Value, mg./kg.	TI_s[e,i]
III	Me	H	H	O	180 (160–230)	9 (7–14)	—	—	—	20	27 (20–45)	—	—
V	Me	H	H	O	176 (140–220)	9 (6–15)	8 (5–16)	60 (45–80)	0.5	20	26 (20–50)	69	2.5
VII	Me	Cl	H	O	190 (170–215)	26 (19–38)	6 (3–10)	35 (12–95)	1.0	7	30 (20–70)	—	—
VIII	Me	Br	H	O	165 (145–190)	38 (27–51)	8 (4–15)	35 (12–95)	1.5	4	29 (20–80)	—	—
IX	Me	H	HO	O	130 (110–140)	18 (15–23)	10 (6–19)	75 (38–100)	1.0	7	—	—	—
X	Me	H	MeO	O	165 (140–195)	9 (6–15)	10 (4–15)	35 (18–62)	0.8	18	—	—	—
XI	Me	H	H_2N	O	35 (28–44)	3 (2–4)	—	—	—	12	29 (20–65)	—	—
XII	Me	Cl	H_2N	O	30 (25–35)	6 (5–7)	4 (2–8)	4[c] (2–8)	0.13	5	26 (20–60)	22	1.4
XV	*n*-Pr	H	H	O	265 (215–285)	34 (27–39)	3 (2–6)	20 (4–120)	0.5	8	28 (20–80)	—	—
XVI	*iso*-Pr	H	H	O	32 (25–36)	10 (5–17)	—	—	—	3	28 (20–80)	—	—
XIX	*iso*-Pr	H	H	H,OH	300 (280–325)	16 (13–20)	18 (9–36)	—	1.8	19	—	—	—
Procaine·HCl					410 (360–465)	18 (12–20)	25 (11–50)	75 —	—	23	20 (20–45)	1500	0.27
Lidocaine·HCl					200 (170–225)	9 (5–14)	26 —	80 —	2.1	22	22 (20–50)	58	3.4
(−)-Cocaine·HCl					68 (55–86)	3 (1.5–4.5)	9 —	14 —	1.0	23	22 (20–55)	66	1.0

[a] Values in parentheses are 95% confidence limits. [b] At highest concentrations used, III–XIX caused severe tissue damage. [c] Convulsions noted in test animals. [d] At highest concentrations used, III–XIX caused local irritation in most instances. [e] Based on the mouse tail test. [f] Time at which one-half of the test animals were still anesthetized. [g] Values in parentheses represent the times at which anesthesia disappeared in the first and last test animals. [h] See the text for the details of determination. [i] The concentration that theoretically would afford local anesthesia of infinite duration. [j] Therapeutic index calculated on the basis of saturation values.

Saturation Values—The method used to determine $T_{1/2}$ at ED_{50} was also applied to previously determined ED_{30} and ED_{70} dose levels. The reciprocal of $T_{1/2}$ at each dose level was plotted against the log of the respective ED_{30}, ED_{50}, and ED_{70} values. The line was extrapolated to infinite time to obtain a value termed the "saturation value," *i.e.*, the amount of drug that theoretically would yield an infinitely long period of local anesthesia.

Nerve Action Potentials—Circumesophageal connections of crayfish, including the median giant axon, were dissected out in lengths of 2 cm. and mounted in a Lucite nerve chamber containing appropriate bathing solution (24). Nerve impulses were induced by electric shocks applied near one end of the axon by means of a pair of platinum electrodes and were recorded with another pair near the other end. The distance between the two sets of electrodes was 13 mm. The diameter of the platinum wire was 100 μ, and the distance between the electrodes in each pair was 1.5 mm. A vaseline sheath was put between each pair of electrodes. Medial giant axons, having diameters ranging from 120–150 μ, were used. The stimulus was amplified *via* a Tektronix 3A3 at 5-v. intensity, with a frequency of 1/sec. and a duration of 0.2 msec. The action potential was recorded on a Tektronix 561 oscilloscope through a Tektronix 3A3 d.c. amplifier. Experiments were conducted at 22°. The photographs from the oscilloscope were taken with a Grass polygraph camera. The negatives obtained were passed through a projector, and conduction time and amplitudes were measured from the projection. The action potential as a control was recorded, and solutions of the drugs were added in increasing concentration. The effects were recorded at regular intervals.

Blood Pressure—Female New Zealand rabbits, weighing 3–5 kg., were anesthetized by injecting 35–40 mg. /kg. of pentobarbital into the marginal ear vein. Following anesthesia, a cannula was inserted into the trachea, and the animals were administered artificial respiration by pump. The femoral vein was cannulated for drug injection. Arterial pressure was measured from the carotid artery by a Statham P-23A transducer connected to a Gilson polygraph.

Electrocardiograms—Lead II records were obtained on the Gilson polygraph using needle electrodes.

LD_{50}—These values were determined with mice (average weight 25–30 g.), using a minimum of 10 mice at three dose levels. The animals were injected subcutaneously in the nape of the neck. After 24 hr., the number of dead animals was used to calculate the LD_{50} using the method of Litchfield and Wilcoxon (25).

RESULTS

No statistically significant differences in potency, toxicity, or duration of effect are evident between V and the dextrorotatory antipode (III) cited in Table I. With the exception of the *p*-H_2N moiety, the LD_{50} values are relatively insensitive to substituents on the aromatic acyloxy unit. Thus, III–X are about 2–3 times more toxic than procaine, about as toxic as lidocaine, and 2–3 times less toxic than cocaine, while XI (*p*-H_2N) and XII (*o*-Cl-*p*-H_2N) are about 13, 6, and 2 times more toxic than the controls, procaine, lidocaine, and cocaine, respectively. On the other hand, the *p*-H_2N group affects LD_{50} more than ED_{50}. The former is 2 times more active than the latter, 6 times more active than procaine (8 times more active on a molar basis), 3 times more active than lidocaine, and equally as active as cocaine.

While the ED_{50} values for the mouse tail assay are sensitive to other aromatic substituents, the ED_{50} values are less sensitive in the other three assays employed. Comparison of the ED_{50} values for one assay with those of the other assays and/or comparison of the derived therapeutic indexes reveal a lack of rank-order coherence. Based upon therapeutic indexes calculated from mouse tail ED_{50}

Table II—Effect of Compounds on Amplitude and Conduction Velocity of the Stimulated Crayfish Nerve Fiber

Drug·HCl	Concentration, m*M*	Change in Amplitude[a], %	Change in Conduction Velocity[a], %	Minutes[b]	Recovery Time, min.
Procaine	10.00	−63	−27	13	5
	50.00	−100	−100	1	8
Lidocaine	10.00	−6	−7	4	1
	50.00	−100	−100	5	2
V	0.50	−6	−4	7.5	2
	1.00	−100	−100	8	2
XII	2.00	−77	−50	20	12
	10.00	−100	−100	4	—[c]

[a] Maximum change from initial values. [b] Time required to produce maximum percent change from initial values. [c] Irreversible block, *i.e.*, no return after washout.

values, there is little essential difference in therapeutic potential between the controls and III, V, X, and XIX, while V (rabbit cornea) and XV (guinea pig wheal, guinea pig sciatic nerve, and rabbit cornea) are inordinately more efficacious than the controls in the tests cited. On the basis of therapeutic indexes calculated with saturation values, V ranks only slightly below lidocaine but 2.5 and 10 times higher than cocaine and procaine, respectively.

As reflected by $T_{1/2}$ values, all compounds so evaluated produce longer lasting effects (26–30 min.) than the controls (20–22 min). At the highest doses used in the initial mouse tail assay, severe tissue damage is produced by the test compounds but not by the controls. The test compounds but not the controls produce slight injection of the vessels of the palpebral conjunctiva and sclera at the highest concentrations used in the rabbit cornea evaluation. However, all eyes appeared normal after 24 hr. No signs of irritation or tissue damage were observed in any assay at the ED_{50} dose. The slopes of dose–response curves are substantially the same for test compounds and controls.

Electrophysiological studies (Table II) show that V produces maximal reversible responses on conduction velocity and amplitude in stimulated crayfish median giant axon preparations at concentrations 50-fold below those required for procaine and lidocaine. While XII and procaine produce similar, reversible, submaximal responses at concentrations of 2 and 10 m*M*, respectively, at 10 m*M* the maximal effects on amplitude and conduction velocity afforded by XII are irreversible, suggesting profound cellular damage or chemical derangement.

In contrast to procaine, which produces no significant vasodepressor response in rabbits at a dose of 2.0 mg./kg., Compound XII, at 1.0 mg./kg., moderately decreases (35%) mean arterial blood pressure for about 4 min. (*cf.*, Table III). The vasodepressor effect is associated with a slight bradycardia and significant alteration in the character of the ECG as reflected by changes in P, QRS, and T waves. At 2 mg./kg., XII caused immediate death in 2 of 3 animals; the remaining rabbit displayed marked lowering of blood pressure (60–70%) associated with extreme bradycardia and marked changes in P, QRS, and T waves.

DISCUSSION AND CONCLUSIONS

Until the recent report of Stubbins *et al.* (26) established the existence of a local anesthetic receptor, support for such a hypothesis, based upon the small differences in diastereoisomeric and antipodal potency and toxicity ratios of amino ester local anesthetics, was tenuous, since small differences in biological effects could result from stereoselective metabolism, adsorption, excretion, transport, *etc.*, rather than from stereoselective receptor binding. The mono-β-chloroethyl analog of lidocaine acts as an alkylating agent, producing lasting local anesthetic action which cannot be washed out. When administered with lidocaine to protect competitively the reaction sites, normal local anesthetic response and washout are observed (26).

Since phospholipids contain the requisite nucleophile, a phosphodiesterate anion, as well as ester dipoles, hydroxyl groups, and nonpolar chains in the esteratic segment, which may interact with both the nonpolar and ester moieties of local anesthetic amino ester cations (27), and since phospholipids have been implicated in local anesthetic response (27–29), it is attractive to think of these, alone or in association with protein, as the "receptor." The facts that phospholipids are optically asymmetric (30) and that the asymmetric esteratic segments are chemically, structurally, and conformationally heterogeneous and relatively remote from the anionic center suggest a possible explanation for the characteristically poor stereochemical discrimination. The combination of phospholipid anion with local anesthetic amino ester cations, reenforced by dipole, hydrogen, van der Waals, and hydrophobic bands, can reasonably be expected to release bound inorganic cations and, in altering phospholipid conformation, effectively bury the anions. This suggests that electrochemical stimulation also effects changes in the membrane conformation of phospholipids and/or lipoprotein, resulting not in the release of normally bound Na cation but in its rapid structural transfer into and across the membrane.

In deciding to investigate the 1-alkyl-3-benzoyl-3-acyloxypiperidine prototype, it was reasoned that the several biological responses of local anesthetics were mediated in chemically different environments by receptors differing in their conformational and/or chemical characteristics. Thus, a change in the conformational population of a drug brought about by steric and/or electrostatic restraints in a molecule more rigid than contemporary local anesthetic amino esters was expected to affect potency and, at the same time, affect selectivity by taking advantage of the conformational adaptabilities of prototype and receptor and/or the topographical and environmental differences of the receptors or drug-bound receptors. Test system dependent variations in antipodal and diastereoisomeric potency ratios, like the observed variations in the rank-order potency of these piperidines from one assay system to the next, reflect subtle differences in permeability, clearance, metabolism, and/or receptor structure, even in the various test tissues. This prototype (I), in providing a higher population of the 4-atom aminoethoxy *gauche*-conformation than is possible with analogs such as procaine, presented a potential opportunity to observe the biological effects of conformational rigidity and homogeneity in local anesthetics.

The data reveal that this prototype produces local anesthesia by characteristic, reversible changes in the nerve membrane. The slopes of the dose–response curves of the test compounds and controls suggest a profound similarity in their mode of action (*27*). In addition, the prototype exhibits the classical side effects, myocardial depression and stimulation of the cerebral cortex as evidenced by convulsions (31, 32), although it does not exhibit antipodal partition of local anesthetic activity (*cf.*, III and V, Table I) in the mouse tail assay. Generally, the effects on potency and toxicity of substituents in the piperidine series do not differ markedly from those observed in the procaine series. What small differences do exist may be attributable to the effect of the benzoyl moiety on the stereochemistry, distribution, and fate of the piperidines.

The empirical formula used as a potential measure of the energy of binding to a receptor is virtually identical for both procaine ($C_{13}H_{21}ClN_2O_2$) and desbenzoyl-XI ($C_{13}H_{19}ClN_2O_2$). The minor role played by the benzoyl unit in determining the local anesthetic potency or energy of binding to the local anesthetic receptor may be reflected in the small difference in potency between XVI and XIX. The 6-carbon diethylaminoethyl unit in procaine and the *N*-methylpiperidine unit in these prototypes apparently subserve the same function in spite of their slight structural difference, since higher alkylation of the piperidine system does not inordinately affect local anesthetic potency (*cf.*, V, XV, and XVI). The generally higher $T_{1/2}$ values for the piperidine analogs compared to the controls may

Table III—Effect of Compound XII on Mean Arterial Blood Pressure of the Rabbit

Dose, mg./kg.	Mean Pressure, mm. Hg Before	Mean Pressure, mm. Hg After	Average Change, mm. Hg	Duration, min.	Heart Rate, beats/min.
0.25	115	115	0	—	215
0.50	120	115	−5	—	215
1.00	105	75	−35	4.0	185
2.00[a]	105	35	−70	8.3	95

[a] Two of three test animals died at this dose level.

be rationalized on the basis of the thermodynamically preferred axial orientation of the smaller of the 3-substituents, the ester group (33), and/or on the basis of the fact that the piperidine analogs are esters of stereochemically more hindered tertiary carbinols. Both structural features tend to depress hydrolysis rates and thus maintain higher concentrations of drug in the biophase. Alternately or additionally, higher $T_{1/2}$ values may reflect greater mechanical affinity for lipid or ionic entities or lower diffusion rates in or about the biophase.

The fact that XI is 8 times more potent than procaine on a molar basis in the mouse tail assay suggests that the *gauche*-conformation, with respect to the 4-atom aminoethoxy moiety in these drugs, has some positive biological significance. The high potency of V and XII relative to procaine in electrophysiological studies on isolated median giant axon of crayfish (Table II) reinforces this argument. Significantly, Sax *et al.* (34) came to the same conclusion. Using di-*p*-nitrophenylphosphoric acid as a model of a phospholipid, they subjected the procaine salt to X-ray crystallographic analysis and found the cation to exist in the *gauche*-conformation about the 4-atom aminoethoxy group. While the solid with its conformational homogeneity may be aphysiological, the established conformations and interactions of the ions may well be biologically relevant. The ester is in the thermodynamically more stable (33) *anti*-conformation with the unshared electron pair and the negative end of the ether oxygen dipole of the ester directed toward the cationic center, while the carbonyl oxygen is flanked by the 2-hydrogens of the carbonyl carbon (*cf.*, II). Structures I and II detail the striking similarity in chemical and stereochemical superimposability patterns of these anesthesiophores.

If the benzoyl group has little or no effect on toxicity also, the 16-fold difference in molar potency between procaine and XI suggests that the *gauche*-conformation has greater biological significance for CNS and cardiac receptors than for peripheral local anesthetic receptors. In effect, the *gauche*-conformation appears to contribute significantly to local anesthetic, cardiac, and CNS activity and acts as a stereochemical determinant, discriminating between local anesthetic receptor on the one hand and in favor of CNS and/or cardiac receptors on the other hand. Amide isosterism in the piperidine series and conformational restriction of the benzoate moiety with respect to the 4-atom aminoethoxy unit in this and in new prototypes are features now under investigation (35).

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 15, 1970, from the *Division of Pharmacology and Biological Sciences and the Laboratory of Medicinal Chemistry, Division of Chemistry, College of Pharmaceutical Sciences, Columbia University, New York, NY 10023*

Accepted for publication December 12, 1970.

Presented in part to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

The authors express appreciation to Dr. James M. Sprague, Dr. P. A. Mattis, and Mr. S. E. McKinney, Merck Sharp and Dohme Research Laboratories, West Point, Pa., for preliminary screening of some compounds; to Harry Grundfest and S. Yamagishi, Department of Neurology, College of Physicians and Surgeons, Columbia University, for advice and for the use of equipment to perform studies on nerve action potentials; and to Dr. M. Sax and Dr. J. Pletcher, Department of Crystallography, University of Pittsburgh, for a timely preprint of their paper on the X-ray crystallographic studies of procaine di-*p*-nitrophenylphosphate.

This investigation was supported by Grant NB-03593 from the U. S. Public Health Service, Bethesda, Md.

* Present address: Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

† Present address: Nease Chemical Co., College Park, PA 16810

‡ Present address: Department of Chemistry, College of Pharmacy, University of Tehran, Tehran, Iran.

§ Author to whom inquiries should be directed.