Keyphrases

3′-Chloro-3-nitrosalicylanilide, tritium labeled—synthesis
Paper chromatography—analysis
Radiochromatography—analysis
Liquid scintillation counting—radioactivity
Lamprey larvae—3′-chloro-3′-nitrosalicylanilide effect

Potential Antiarrhythmic Agents I

Synthesis and Pharmacological Evaluation of Some Piperazine and Ethylenediamine Analogs of Procaine Amide

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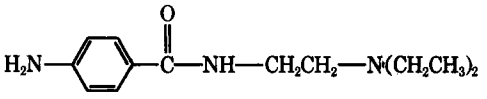
Fourteen 1-alkyl-4-benzoyl-, 1-alkyl-4-*p*-substituted benzoylpiperazine hydrochlorides and eight *N,N′*-dibenzoyl-, *N,N′*-di(*p*-substituted benzoyl)-*N*-alkylethylenediamines were synthesized as procaine amide analogs. These compounds were tested for their effect on the effective refractory period of isolated guinea pig atria. 1-*n*-Propyl-4-benzoylpiperazine hydrochloride, 1-*n*-butyl-4-benzoylpiperazine hydrochloride, 1-*n*-propyl-4-*p*-methoxybenzoylpiperazine hydrochloride, 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride, 1-isobutyl-4-*p*-chlorobenzoylpiperazine hydrochloride, 1-*n*-pentyl-4-*p*-chlorobenzoylpiperazine hydrochloride, and the eight *N,N′*-dibenzoyl-, *N,N′*-di(*p*-substituted benzoyl)-*N*-alkylethylenediamines were further screened for antiarrhythmic activity in cats. Only 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride showed some degree of protection against aconitine-induced cardiac arrhythmias.

THE RELATIONSHIP between the structure and the antiarrhythmic activity of procaine amide [*N*-(2-diethylaminoethyl)-*p*-aminobenzamide, I] has not been thoroughly studied. A few



I

workers, however, have studied the alteration of antiarrhythmic activity caused by various substituents on the benzene ring. Clinton *et al.* (1) reported that several of the higher members of the *N*-(2-diethylaminoethyl)-4-amino-2-alkoxybenzamides, in particular the 2-hexoxy derivative, showed outstanding properties as antiarrhythmic agents. Reisner and Cordasco (2) synthesized 2-chloroprocaine amide and found that this compound was approximately four times as potent as procaine amide in blocking atrial fibrillation in dogs. Libonati and Segre (3) studied the antiarrhythmic activity of *N*-(2-diethylaminoethyl)-*p*-4-hydroxybutylbenzamide and *N*-(2-diethylaminoethyl)-*p*-isopropylbenzamide and reported that these compounds showed an activity of 75 and 10 times, respectively, that of procaine amide. Similar compounds, such as *N*-(2-diethylaminoethyl)benzamide and *N*-(2-diethylaminoethyl)-*p*-methoxybenzamide, have also been evaluated for antiarrhythmic activity (4). The former compound was found to have a lower toxicity but the same potency as procaine amide and the latter compound to have a higher potency and toxicity than procaine amide.

The first systematic synthesis of procaine amide analogs was reported by Thyrum and Day (5). In the series of compounds they prepared, the following showed significant antiarrhythmic activity when tested on isolated

Received July 17, 1968, from the Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada.
Accepted for publication September 3, 1968.
Grateful acknowledgment is made to the National Research Council of Canada for financial assistance and to Dr. L. P. Chénier, Miss Joan Maxwell, and Mr. Leo Fleming of Frank W. Horner Ltd., Montreal, Quebec, Canada for performing the toxicity studies and testing some of the compounds for antiarrhythmic activity.
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TABLE I—1-ALKYLPIPERAZINES

Compd.	Alkyl	Formula	Yield, %	B.p., °C./mm. Found	B.p., °C./mm. Reported
II	$-CH_2CH_3$[a]	$C_6H_{14}N_2$	42	57–60/40	64/50 (6)
III	$-CH_2CH_2CH_3$	$C_7H_{16}N_2$	48	75–77/37	65/20 (6)
IV	$-CH_2CH_2CH_2CH_3$	$C_8H_{18}N_2$	42	97–100/40	84/10 (6)
V	$-CH_2CH(CH_3)_2$	$C_8H_{18}N_2$	57	88–90/47	78–80/13 (7)
VI	$-CH_2CH_2CH_2CH_2CH_3$[b]	$C_9H_{20}N_2$	58	116/48	105–110/9 (8)

[a] M.p. of the dipicrate 245.8–246.0° dec., reported m.p. 249° dec. (9). [b] M.p. of the di-HCl 265.0–266.8° dec., reported m.p. 267–269° dec. (8).

rabbit heart: *N*-[2-(2-indolinyl)ethyl]-*p*-aminobenzamide, *N,N*-[bis-2(*p*-aminobenzamido)ethyl]-*n*-butylamine, *N*-(2-piperidinoethyl)-*p*-aminobenzamide, and *N*-[2-(*N*-methylanilino)ethyl]-*p*-aminobenzamide. The latter compound was reported to have a potency 15 times that of procaine amide.

It would thus appear that studies of the structure-activity relationship of procaine amide so far reported have been concerned with replacement of the aromatic amino group in the molecule by other groupings, substitution on the benzene ring, and modification of the diethyl substituent on the terminal tertiary nitrogen atom. The effects on activity caused by joining the amide nitrogen and the terminal tertiary amine nitrogen together by cyclization or conversion of the terminal tertiary amine nitrogen to a tertiary amide nitrogen in the procaine amide molecule have not been examined. The present work set out to study these molecular varients in an attempt to provide additional information regarding the structure-activity relationship of procaine amide and to prepare some potential antiarrhythmic agents. It was also the intention of the authors to attempt to map a receptor for the antiarrhythmic agents.

EXPERIMENTAL

Chemical Synthesis

The synthesis of the compounds was initiated by treating excess piperazine or ethylenediamine with the various alkyl halides to produce the 1-alkylpiperazines or *N*-alkylethylenediamines. These monoalkylated piperazines and ethylenediamines were then allowed to react with benzoyl, anisoyl, *p*-chlorobenzoyl, or *p*-nitrobenzoyl chloride to yield the desired compounds. The 1-alkyl-4-*p*-nitrobenzoylpiperazines and *N,N'*-di(*p*-nitrobenzoyl)-*N*-alkylethylenediamines were reduced to the corresponding amino compounds with iron and hydrochloric acid.

Melting points were taken with a Thomas-Hoover capillary melting-point apparatus and are corrected. Elemental analyses were performed by Weiler and Strauss, Oxford, England.

1-Alkylpiperazines (Table I)—Anhydrous piperazine (50.0 g., 0.56 mole) was dissolved in absolute ethanol (150 ml.). To this solution was added the alkyl bromide (0.20 mole). The reaction mixture was refluxed for 10–12 hr. and then neutralized by sodium carbonate (0.25 mole). The ethanol was then removed by distillation, and after the addition of ice cold water (*ca.* 300 ml.), the reaction mixture was extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and then filtered. After the removal of the solvent by distillation *in vacuo*, the products were distilled under reduced pressure.

N-Alkylethylenediamines (Table II)—The above procedure for preparing the 1-alkylpiperazines was followed, with the exception that 4 parts of anhydrous ethylenediamine were used to react with 1 part of an alkyl bromide.

1-Alkyl-4-benzoyl- and 1-Alkyl-4-p-substituted Benzoylpiperazine Hydrochlorides (Table III)—The 1-alkylpiperazine (0.2 mole) was dissolved in a mixture of dry benzene and anhydrous ether (1:1, 150 ml.). The acyl chloride (0.2 mole) in dry benzene (30 ml.) was added dropwise to the solution kept at a temperature of 0–5°. Stirring was continued for 90 min at 10° after the addition was complete. The precipitate was then collected, washed with a small amount of anhydrous ether, and recrystallized from 2-propanol.

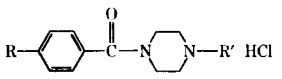
N,N'-Dibenzoyl- and N,N-Di(p-substituted benzoyl)-N-alkylethylenediamines (Table IV)—To a mixture of 10% sodium hydroxide (40 ml.) and *N*-alkylethylenediamine (0.05 mole) in chloroform (100 ml.) was slowly added a chloroform solution of the acyl chloride (0.10 mole). The resulting mixture was kept at a temperature of 0–5°. After the addition was complete, stirring was allowed to continue for 4–5 hr., while maintaining the temperature below 10°. The chloroform layer was separated and the

TABLE II—*N*-ALKYLETHYLENEDIAMINES

Compd.	Alkyl	Formula	Yield, %	B.p., °C./mm. Found	B.p., °C./mm. Reported
VII	$-CH_2CH_2CH_3$[a]	$C_5H_{14}N_2$	40	52–56/38	153–154/749 (10)
VIII	$-CH(CH_3)_2$	$C_5H_{14}N_2$	57	134/724	137–138/752 (10)
IX	$-CH_2CH_2CH_2CH_3$[b]	$C_6H_{16}N_2$	50	74–76/37	78–80/25 (10)
X	$-CH_2CH(CH_3)_2$	$C_6H_{16}N_2$	47	72–73/51	—
XI	$-CH_2CH_2CH_2CH_2CH_3$[c]	$C_7H_{18}N_2$	56	98–100/51	135–140/90 (11)

[a] M.p. of the di-HCl 206.0° dec., reported m.p. 204–205° dec. (10). [b] M.p. of the di-HCl 230.5–231.8° dec., reported m.p. 230° dec. (10). [c] M.p. of the di-HCl 235.4–236.8° dec., reported m.p. 236–237° dec. (12).

TABLE III—1-ALKYL-4-BENZOYL- AND 1-ALKYL-4-*p*-SUBSTITUTED BENZOYLPIPERAZINE HYDROCHLORIDES

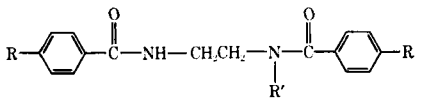


Compd.	R	R′	Formula	M.p., °C.	Yield, %		Anal., % Calcd.	Found
XII	—H	—CH_2CH_3	$C_{13}H_{19}ClN_2O$	243.0–244.5 dec.	48	C,	61.29	61.34
						H,	7.52	7.42
						N,	11.00	10.81
XIII	—H	—$CH_2CH_2CH_3$	$C_{14}H_{21}ClN_2O$	214.0–214.8 dec.	95	C,	62.55	62.60
						H,	7.88	7.74
						N,	10.43	10.10
XIV	—H	—$CH_2CH_2CH_2CH_3$	$C_{15}H_{23}ClN_2O$	224.0–224.9 dec.	88	C,	63.70	63.53
						H,	8.20	8.11
						N,	9.91	10.02
XV	—OCH_3	—CH_2CH_3	$C_{14}H_{21}ClN_2O_2$	190–192.5 dec.	93	C,	59.04	59.00
						H,	7.43	7.40
						N,	9.84	10.00
XVI	—OCH_3	—$CH_2CH_2CH_3$	$C_{15}H_{23}ClN_2O_2$	218.0–220.0 dec.	84	C,	60.29	60.19
						H,	7.76	7.71
						N,	9.38	9.62
XVII	—OCH_3	—$CH_2CH_2CH_2CH_3$	$C_{16}H_{25}ClN_2O_2$	192.0–193.0	93	C,	61.43	61.53
						H,	8.06	7.98
						N,	8.96	9.00
XVIII	—Cl	—CH_2CH_3	$C_{13}H_{18}ClN_2O$	240–243.0 dec.	76	C,	53.99	54.06
						H,	6.27	6.27
						N,	9.69	9.58
XIX	—Cl	—$CH_2CH_2CH_3$	$C_{14}H_{20}Cl_2N_2O$	249.0–250 dec.	92	C,	55.45	55.35
						H,	6.65	6.70
						N,	9.24	9.40
XX	—Cl	—$CH_2CH_2CH_2CH_3$	$C_{15}H_{22}Cl_2N_2O$	228.5–229.2 dec.	78	C,	56.78	56.76
						H,	6.99	7.06
						N,	8.83	8.58
XXI	—Cl	—$CH_2CH(CH_3)_2$	$C_{15}H_{22}Cl_2N_2O$	240.0–241.6 dec.	94	C,	56.78	56.80
						H,	6.99	7.09
						N,	8.83	8.92
XXII	—Cl	—$CH_2CH_2CH_2CH_2CH_3$	$C_{16}H_{24}Cl_2N_2O$	223.6–225.0 dec.	93	C,	58.01	58.07
						H,	7.30	7.41
						N,	8.46	8.29
XXIII	—NO_2	—CH_2CH_3	$C_{13}H_{18}ClN_3O_3$	about 289 dec.	49	C,	52.09	52.41
						H,	6.05	6.10
						N,	14.02	14.49
XXIV	—NH_2	—CH_2CH_3[a]	$C_{13}H_{19}N_3O$	137.4–138.1	56	C,	66.92	66.91
						H,	8.21	8.19
						N,	18.01	17.86
XXV	—NH_2	—$CH_2CH_3 \cdot 2H_2O$	$C_{13}H_{24}ClN_3O_3$	foams 180	45	C,	51.06	51.01
						H,	7.91	7.73
						N,	13.74	13.95
XXVI	—NO_2	—$CH_2CH_2CH_3$	$C_{14}H_{20}ClN_3O_3$	277.2–280.5 dec.	94	C,	53.59	53.54
						H,	6.43	6.41
						N,	13.39	13.43
XXVII	—NH_2	—$CH_2CH_2CH_3 \cdot H_2O$[a]	$C_{14}H_{23}N_3O_2$	96.2–102.0	81	C,	63.36	63.22
						H,	8.74	8.74
						N,	15.84	15.83
XXVIII	—NH_2	—$CH_2CH_2CH_3 \cdot 2H_2O$	$C_{14}H_{26}ClN_3O_3$	above 300	76	C,	52.57	52.46
						H,	8.20	8.24
						N,	13.14	13.10
XXIX	—NO_2	—$CH_2CH_2CH_2CH_3$	$C_{15}N_{21}ClN_3O_3$	256.0–256.6 dec.	87	C,	54.96	55.00
						H,	8.87	8.92
						N,	12.82	12.90
XXX	—NH_2	—$CH_2CH_2CH_2CH_3$[a]	$C_{15}H_{23}N_3O$	103.0–103.5	53	C,	68.93	69.13
						H,	8.87	8.92
						N,	16.08	15.72
XXXI	—NH_2	—$CH_2CH_2CH_2CH_3 \cdot H_2O$[b]	$C_{15}H_{27}ClN_3O_2$	above 280	45	C,	51.14	51.11
						H,	7.73	7.71
						N,	11.9	12.35

[a] Free base. [b] Dihydrochloride salt.

aqueous layer was washed with chloroform. The combined chloroform solutions were washed with water, dried over anhydrous sodium sulfate, and the solvent removed under vacuum. Ether or *n*-hexane was stirred into the residue until a white solid was obtained. The product was removed by filtration, washed with ether or *n*-hexane, and recrystallized from acetone and *n*-hexane.

TABLE IV—*N,N'*-DIBENZOYL- AND *N,N'*-DI(*p*-SUBSTITUTED BENZOYL)-*N*-ALKYLETHYLENEDIAMINES



Compd.	R	R'	Formula	M.p., °C.	Yield, %		Anal., % Calcd.	Anal., % Found
XXXII	$-NO_2$	$-CH_2CH_2CH_3$	$C_{19}H_{20}N_4O_6$	190.0–190.5	64	C,	56.99	57.25
						H,	5.04	4.86
						N,	13.99	14.12
XXXIII	$-NH_2$	$-CH_2CH_2CH_3$	$C_{19}H_{24}N_4O_2$	89.0–92.0	77	C,	67.03	67.50
						H,	7.11	7.11
						N,	16.46	16.10
XXXIV	$-NO_2$	$-CH_2CH(CH_3)_2$	$C_{20}H_{22}N_4O_6$	178.0–178.9	81	C,	57.96	57.95
						H,	5.35	5.69
						N,	13.52	13.58
XXXV	$-NH_2$	$-CH_2CH(CH_3)_2$	$C_{20}H_{26}N_4O_2$	156.0–156.6	93	C,	67.77	67.95
						H,	7.40	7.41
						N,	15.81	15.71
XXXVI	—Cl	$-CH(CH_3)_2$	$C_{19}H_{20}Cl_2N_2O_2$	184.2–184.9	66	C,	60.16	60.18
						H,	5.32	5.48
						N,	7.39	7.35
XXXVII	—Cl	$-CH_2CH_2CH_2CH_3$	$C_{20}H_{22}Cl_2N_2O_2$	111.8–112.5	62	C,	61.07	60.97
						H,	5.64	5.68
						N,	7.12	7.09
XXXVIII	$-OCH_3$	$-CH_2CH_2CH_3$	$C_{21}H_{26}N_2O_4$	125.9–127.0	87	C,	68.08	68.13
						H,	7.08	7.18
						N,	7.56	7.41
XXXIX	$-OCH_3$	$-CH_2CH_2CH_2CH_3$	$C_{22}H_{28}N_2O_4$	109.4–110.0	91	C,	68.73	68.74
						H,	7.34	7.37
						N,	7.29	7.47
XL	—H	$-CH_2CH(CH_3)_2$	$C_{20}H_{24}N_2O_2$	144.6–145.6	42	C,	74.04	73.68
						H,	7.46	7.50
						N,	8.64	8.65
XLI	—H	$-CH_2CH_2CH_2CH_3$	$C_{21}H_{26}N_2O_2$	105.0–106.0	47	C,	74.52	74.26
						H,	7.74	7.49
						N,	8.27	8.58

1-Alkyl-4-p-aminobenzoylpiperazines and Their Hydrochlorides (Table III)—A suspension of powdered iron (0.30 mole) in water (*ca.* 80 ml.) and concentrated hydrochloric acid (2 ml.) was heated to 85° and a warm aqueous solution of the 1-alkyl-4-*p*-nitrobenzoylpiperazine hydrochloride (0.05 mole) was added in small portions with stirring. The mixture was stirred and heated for 2 hr. at 75–80° and filtered while hot. The black residue was washed with hot water and the filtrate chilled, filtered and made alkaline with 10% sodium carbonate solution. The solution was then extracted with chloroform. The combined chloroform extracts were washed with water and dried over anhydrous sodium sulfate overnight. After removal of the solvent, the residue was dissolved in a small amount of acetone and *n*-hexane was added until the solution became cloudy. The solid 1-alkyl-4-*p*-aminobenzoylpiperazines were later collected and recrystallized from acetone and *n*-hexane, or from ethanol.

The hydrochlorides were prepared by passing dry hydrogen chloride gas into a solution of the base in anhydrous methanol and recrystallized from methanol and ether.

N,N' - Di(p - aminobenzoyl) - N - alkylethylenediamines (Table IV)—These compounds were prepared according to the method described by Clinton *et al.* (13) for the synthesis of ethyl 2-benzoyloxy-4-aminobenzoate.

Pharmacological Testing

The pharmacological testing was carried out in two stages. The first stage involved a determination of the relative activities of all the compounds synthesized on the effective refractory period of isolated electrically driven guinea pig atria. This was done by measuring the changes induced by a compound in the maximum rate of stimulation (MSR) that the suspended atria would follow with a 1:1 stimulus–contraction ratio (Table V). The rationale for this determination is based on the fact that prolongation of the refractory period of cardiac muscle is an important factor in terminating and preventing the occurrence of arrhythmias. The method of determination was that reported by MacLeod and Reynolds (14) and was essentially as follows: after the atria were properly mounted for recording of contractile activity in the tissue bath, they were driven by a Grass model S4C stimulator at progressively increasing rates with frequency increments of 12 shocks per minute for 20-sec. intervals until they failed to follow the stimulus; this was manifested in the recording as a dropped beat followed by a contraction of greater than normal amplitude. Recording of contractile activity was done by means of a force displacement transducer on a physiograph. The rate at which the atria failed to follow the stimulus was taken as the MSR. At least four control determinations were carried out, depending upon their reproducibility. The

TABLE V—EFFECT OF COMPOUNDS ON THE MAXIMUM STIMULATION RATES (MSR) OF ISOLATED GUINEA PIG ATRIA[a]

Compd.	Concn.	MSR, Shocks/min. ± SD		Average % Depression, MSR
		Control	Compd.	
XII	1:5,000	416±6.9	396±12.2	No effect
XIII	1:5,000	525±12.2	356±6.9	32.2±1.3
	1:10,000	417±24.9	360±9.8	13.7±2.3
XIV	1:5,000	438±14.7	324±12.0[b]	26.0±2.7
	1:10,000	491±7.5	357±14.8	27.3±3.1
XV	1:5,000	354±12.6	363±15.1	No effect
XVI	1:5,000	354±12.6	[c]	—
	1:10,000	453±12.6	430±8.7	5.1±1.9
XVII	1:10,000	343±4.6	[c]	—
	1:50,000	324±12.6	[c]	—
XVIII	1:5,000	431±14.4	416±6.9	No effect
XIX	1:5,000	375±6.0	[c]	—
	1:10,000	412±27.3	304±25.0	26.2±6.1
XX	1:5,000	325±12.0	320±6.9	No effect
XXI	1:10,000	354±12.6	[c]	—
	1:100,000	416±6.9	[c]	—
XXII	1:50,000	427±25.8	[c]	—
	1:100,000	375±7.5	[c]	—
XV	1:5,000	312±3.2	310±10.2	No effect
XXVIII	1:5,000	371±19.6	336±13.9	9.7±3.2
XXXI	1:5,000	410±6.9	268±6.9	34.6±1.7
	1:10,000	381±6.0	304±6.9	20.2±1.8
XXXIII	1:100,000	424±12.6	[c]	—
XXXV	1:100,000	312±6.9	[c]	—
XXXVI	1:100,000	377±6.6	[c]	—
	1:200,000	389±12.0	[d]	—
XXXVII	1:100,000	438±11.5	[c]	—
	1:200,000	354±6.9	[d]	—
XXXVIII	1:100,000	354±12.6	[d]	—
XXXIX	1:100,000	381±12.0	[c]	—
XL	1:100,000	461±7.5	460±8.7	No effect
XLI	1:200,000	336±7.5	[c]	—
Procaine amide hydrochloride	1:5,000	443±15	318±25.5	28.2±5.2
	1:10,000	440±21	376±13.9	14.5±3.1
	1:50,000	536±7.5	495±15.0	7.7±2.8
	1:100,000	499±7.5	490±6.9	No effect

[a] Unless otherwise indicated, data in this table were obtained at the 15-min. interval. [b] Calculation based on the 10-min interval, because atria became too depressed to be measured at the 15-min. interval. [c] Atria stopped beating. [d] Atria too depressed to be measured.

control value was taken as the average of these determinations. The test compound was then added to the tissue bath and the MSR was determined at 5-, 10-, and 15-min. intervals. Determinations were repeated three times after the test compound was washed out of the tissue bath and the MSR had returned to approximately control levels. The piperazine hydrochlorides were dissolved in demineralized water, and the ethylenediamine compounds in 95% ethanol so that 1 ml. of the resulting solution added to the tissue bath gave the desired concentration.

The second stage of the pharmacological testing was the evaluation of some of the compounds for their ability to prevent or to terminate arrhythmias in cats (Table VI). The method as provided by Frank W. Horner Ltd. for inducing the arrhythmias was local application of aconitine nitrate solution (25%) on the right atrium of the cat. A somewhat different procedure was employed in testing Compound XIV for antiarrhythmic activity in this laboratory. The arrhythmias were induced by petroleum ether and adrenaline (14). After a control arrhythmia produced by adrenaline following petroleum ether inhalation, the cat was then treated with the compound at doses varying between 2 and 24 mg./kg. The petroleum ether–adrenaline challenge was repeated within 15 min. of the injection of the compound. Electrocardiogram and blood pressure recordings were taken before petroleum ether inhalation had begun and continuously from just before adrenaline injection to the end of the arrhythmias. Procaine amide hydrochloride was employed as the standard.

RESULTS AND DISCUSSION

In order to determine whether any effect on the antiarrhythmic activity of procaine amide was produced by joining the amide nitrogen and the terminal tertiary amine nitrogen in the molecule by cyclization, 1-ethyl-4-*p*-aminobenzylpiperazine hydrochloride dihydrate (XXV) was synthesized by inducing a piperazine ring. It has been reported by Ellis and Sivertsen (16) that some *o*-alkylated benzhydrylpiperazines showed considerable antiarrhythmic activity. Another piperazine derivative, hydroxyzine has been introduced in clinical use for treatment of arrhythmias (17). This appears to indicate that the piperazine ring might be making a significant

TABLE VI—ANTIARRHYTHMIC ACTIVITY OF SOME COMPOUNDS[a]

Compd.	LD_{50}[b] mg./kg.	Heart Frequency Before Compd. after Aconitine per min.	Dose, mg./kg. I.V.	Heart Frequency After Compd. per min.
XIII	240	Atrial = 900 Vent. = 300	20	Atrial = 240 Vent. = 240
XVI	218	Atrial >1,000 Vent. = 600	20	Atrial = 500 Vent. = 150
XIX	60	Atrial = 400 Vent. = 200	20	Atrial = 200 Vent. = 200
XIX	60	Atrial = 600 Vent. = 200	20	[c]
XIX	60	Atrial >1,000 Vent. = 200	20/10/10[d]	Atrial = 200 Vent. = 200
XIX	60	Atrial >1,000 Vent. = 200	20	Atrial = 200 Vent. = 200
XXI	360	Atrial >1,000 Vent. = 200	20	Atrial = 280 Vent. = 140
XXVIII	142	Atrial = 600 Vent. = 300	20	Atrial = 500 Vent. = 250
XXXI	128	Atrial = 600 Vent. = 300	20	Atrial = 600 Vent. = 300
Quinidine sulfate	235	Atrial >1,000 Vent. = 200	10	Atrial = 200 Vent. = 200
Quinidine sulfate	235	Atrial >1,000 Vent. = 180	10	Atrial = 150 Vent. = 150

[a] Data supplied by Frank W. Horner Ltd. [b] Calculated by the method of Litchfield and Wilcoxon (15) and based on 3-day observations. [c] Cardiac arrest. [d] Consecutive dose.

contribution to the antiarrhythmic activity of these compounds. However, according to the data listed in Table V, Compound XXV showed no activity in reducing the MSR of isolated guinea pig atria at a concentration of 1:5,000, whereas procaine amide hydrochloride at this concentration reduced the MSR by 28.2 ± 5.2%. Since this compound did not prolong the refractory period of isolated guinea pig atria, it was considered to be devoid of antiarrhythmic activity. Therefore, joining the amide nitrogen and the aliphatic amine nitrogen together in the procaine amide molecule by cyclization appears to reduce or eliminate its antiarrhythmic activity.

Compound XXV was modified by replacing the *N*-ethyl side chain by a propyl group (XXVIII) or a butyl group (XXXI). Although the resulting compounds were capable of reducing the MSR (Table V), they failed to show any antiarrhythmic property when tested in intact animals (Table VI). Therefore, no further attempt was made to modify the *N*-alkyl chain length of Compound XXV.

Other modifications were carried out in which the aromatic amino substituent was replaced by a hydrogen, methoxy, or chloro group. Accordingly, Compounds XII–XX were synthesized. From the data in Table V it is noted that Compounds XIII, XIV, XVI, and XIX exhibited an effect on the refractory period of atrial muscle and for this reason they were tested for antiarrhythmic activity in intact animals. The results summarized in Table VI show that only Compound XIX was found to possess antiarrhythmic activity in these experiments. In three out of four trials with this material, a sinus rhythm of normal frequency was established within 2 min. and lasted from 40 min. to 3 hr. In the fourth experiment cardiac arrest brought about the death of the animal. Control cats treated with quinidine also showed a sinus rhythm maintained from 2 to 4 hr. It appears from these experiments that the antiarrhythmic activity of Compound XIX is approximately half that of quinidine. Since procaine amide hydrochloride is less potent than quinidine on a weight basis (18), Compound XIX may be considered to have an activity similar to that of procaine amide hydrochloride.

Compound XIV was investigated to determine whether it provided protection against arrhythmias induced by the combined use of adrenaline and petroleum ether in the cat. These tests showed that this compound exhibited no antiarrhythmic activity at a dose of 24 mg./kg. The dosage for procaine amide hydrochloride for preventing the occurrence of arrhythmias induced by the same technique was 8 mg./kg.

Compounds XXI and XXII were prepared following the detection of antiarrhythmic activity in Compound XIX. These two substances showed strong depressant action on the force and rate of contraction of isolated guinea pig atria even at a concentration of 1:100,000. The atria were usually completely arrested at this concentration and therefore the effect of Compounds XXI and XXII on the MSR could not be determined. Nevertheless, Compound XXI was tested for antiarrhythmic activity in intact animals and was found to be inactive.

A survey of the antiarrhythmic agents showed that the great majority of them possess a tertiary amine nitrogen in the molecule. Conn and Luchi (19) pointed out that the tertiary amine nitrogen in quinidine played an important role in the effectiveness of this drug. However, in the antiarrhythmic agent diphenylhydantoin (20) only two amide nitrogens are present in the molecule. Thyrum *et al.* (21) found that substitution of an aromatic group for one of the ethyl groups at the tertiary amine nitrogen of procaine amide gave highly active compounds. According to these workers, the basis for the increased activity of the aromatic substituents was probably due to the additional hydrophobic binding provided by the aromatic rings. Such additional binding would improve the facility for interaction between the drug and the receptor. Therefore, the

introduction of a benzoyl group on the tertiary amine nitrogen in procaine amide, as typified by the ethylenediamine compounds in this investigation, should be expected to produce the same effect. However, this alteration also changes the character of that nitrogen to an amide from an amine. Consequently, it is no longer possible for the molecule to be protonated at physiological pH. Any information with regard to the relationship between the activity and the ability of the molecule to be protonated at physiological pH would be valuable in determining whether an anionic site exists at the receptor site. The presence of a charged group on an active compound suggests a counter charged group in the receptor.

In light of this, it was considered of interest to synthesize some ethylenediamine analogs of procaine amide in which the tertiary amine nitrogen in the parent compound was replaced by a tertiary amide nitrogen and to study the effect of this modification on the antiarrhythmic activity. Consequently, Compounds XXXIII, XXXV–XLI were prepared. As shown in Table V these compounds did not reduce the MSR to any measurable extent at the concentrations used. On the other hand, these compounds exhibited a remarkable depressant action on the force and rate of contraction of the atria, even at a concentration as low as 1:100,000. At this concentration, the atrial beat was usually completely inhibited, but the activity returned shortly after several washings. In one instance, however, a concentration of 1:20,000 of Compound XXXVII was found to completely abolish the contraction of the atria despite repeated washings.

To be certain that the various effects on the isolated atria were due to the compounds themselves and not to the 95% ethanol which was employed as the solvent, control experiments with 95% ethanol were conducted. Results showed that the small quantity (0.3 ml.) of ethanol used had no effect on either MSR or force of contraction.

Despite the fact that the ethylenediamine analogs (Compounds XXXIII, XXXV–XLI) did not exhibit any activity in depressing the MSR of isolated guinea pig atria, they were tested for antiarrhythmic activity in intact animals. The results of these experiments showed that compounds XXXIII, XXXV–XLI were absolutely without antiarrhythmic activity. Therefore, it would appear that the terminal amine nitrogen in procaine amide is essential for activity.

When the effective refractory period studies demonstrated that some compounds had marked depressant action on the contraction of isolated guinea pig atria, it was considered desirable to determine whether the nature of the depressant action was due to parasympathomimetic activity. If these compounds did act by this mechanism, then a parasympatholytic substance, such as atropine, would block the depressant action. For this reason, a solution of atropine sulfate was added to the tissue bath to give a concentration of 3 mcg. of the substance and after 5 min., the test compounds were administered. The experiments were repeated three times and on two different atria. The compounds chosen for the test were Compounds XXI and XXXIX, because both showed strong depressant activity (see Table V). The concentration used was 1:100,000 for both compounds. Results of the experiments indicated that these two compounds did not appear to possess parasympathomimetic activity, since the atropine sulfate did not prevent their depressant effect in the slightest manner. The same concentration (3 mcg.) of atropine sulfate, however, was found to block the depressant action of acetylcholine in a concentration of 1:100,000. These observations confirmed that those compounds which depressed atria did so by a direct action on the muscle.

The results of this investigation have shown that the tertiary amine nitrogen in procaine amide must not be changed to an amide nitrogen, thus indicating the possibility of an anionic site on the receptor surface. This view is supported by the fact that such a tertiary amine nitrogen is found in agents of proven antiarrhythmic activity, such as quinidine, lidocaine (22), antazoline (23), hydroxyzine (17), amotriphene (24), pronethalol (25), and Compound XIX of this work. These agents can be protonated at physiological pH and therefore can bind to the anionic site on the receptor by ionic bonding. Another surface feature that can be present on the receptor is a flat area which allows binding with the area of aromaticity provided by the aromatic ring of the antiarrhythmic agents through hydrophobic or van der Waals' forces. Such an area of aromaticity is found in all antiarrhythmic agents mentioned above.

In order to establish the relative position of the anionic site to the flat area on the receptor surface, a Fisher-Hirschfelder-Taylor molecular model was prepared for all the antiarrhythmic agents cited above. It was found that despite the differences in the chemical structures, all these compounds can adopt a common orientation in which the tertiary amine nitrogen is approximately 4.5–5.0 Å. away from the center and 3.0–3.5 Å. above the plane of the area of aromaticity. The configuration considered here may not be the most stable, but one which could occur on the receptor.

This proposed receptor cannot explain why diphenylhydantoin has antiarrhythmic activity, since this molecule apparently can only bind to the flat area and not to the anionic site of the proposed receptor due to its lack of a basic nitrogen. It is probable that diphenylhydantoin exerts its antiarrhythmic action through a different mechanism.

SUMMARY

1. In the procaine amide molecule conversion of the tertiary amine nitrogen to an amide nitrogen causes complete loss of activity, as indicated by the ethylenediamine analogs (Compounds XXXIII, XXXV–XLI).

2. Converting the amide nitrogen and the terminal tertiary amine nitrogen in procaine amide to a piperazine ring produced a compound, namely, 1-ethyl-4-*p*-aminobenzoylpiperazine hydrochloride dihydrate, which has no antiarrhythmic activity.

3. The ethylenediamine analogs (Compounds XXXIII, XXXV–XLI) showed marked depressant activity on the force and rate of contraction of isolated guinea pig atria. Some 1-alkyl-4-*p*-chloro- and 1-alkyl-4-*p*-methoxybenzoylpiperazine hydrochlorides show similar effect. This depressant activity does not appear to involve the autonomic nervous system and is likely the result of a direct depressant action on the atrial muscle.

4. 1-*p*-Propyl-4-*p*-chlorobenzoylpiperazine hy-

drochloride was found to have antiarrhythmic activity in cats. It appears to have a potency similar to that of procaine amide hydrochloride. Replacement of the chloro substituent in 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride by a hydrogen, methoxy, or amino group, or the *n*-propyl group by other alkyl groups abolished the activity.

5. A receptor for antiarrhythmic agents has been proposed to consist of an anionic site and a flat area. The anionic site is believed to be approximately 4.5–5.0 Å. away from the center and 3.0–3.5 Å. above the plane of the flat area.

REFERENCES

(1) Clinton, R. O., Laskowski, S. C., Salvador, V. J., Bate, H. G., and Carroll, P. M., *J. Am. Chem. Soc.*, **79**, 2285(1957).
(2) Reisner, P. B., and Cordasco, M. G., *J. Org. Chem.*, **23**, 1403(1958).
(3) Libonati, M., and Segre, G., *Arch. Ital. Sci. Farmacol.*, **9**, 170(1959); through *Chem. Abstr.*, **54**, 1749*e*(1960).
(4) Hazard, R., Giudicelli, R., Thuillier, G., and Beauvallet, M., *Compt. Rend. Soc. Biol.* **147**, 755(1953); through *Chem. Abstr.*, **48**, 2907*d*(1954).
(5) Thyrum, P., and Day, A. R., *J. Med. Chem.*, **8**, 107 (1965).
(6) Nakajima, K., *Bull. Chem. Soc. Japan*, **34**, 651(1961); *ibid.*, **34**, 655(1961).
(7) Wall, L. P., British pat. 834,300; through *Chem. Abstr.*, **54**, 24822*h*(1960).
(8) Lutz, R. E., Bailey, S. P., and Shearer, N. H., Jr., *J. Am. Chem. Soc.*, **68**, 2224(1956).
(9) Ishiguro, K., Kitamura, E., Matsumura, M., and Ogawa, H., *J. Pharm. Soc. Japan*, **75**, 674(1955); through *Chem. Abstr.*, **50**, 3461*i*(1956).
(10) O'Gee, R. A., and Woodburn, H. M., *J. Am. Chem. Soc.*, **73**, 1370(1951).
(11) King, J. A., and McMillan, F. H., *ibid.*, **68**, 1774 (1946).
(12) Benko, C., and Tisler, M., *Croa. Chem. Acta*, **30**, 243(1958); through *Chem. Abstr.*, **54**, 2221*i*(1960).
(13) Clinton, R. O., Laskowski, S. C., Salvador, U. J., and Wilson, M., *J. Am. Chem. Soc.*, **73**, 3674(1951).
(14) MacLeod, D. P., and Reynolds, A. K., *Can. J. Physiol. Pharmacol.*, **42**, 431(1964).
(15) Litchfield, T. J., Jr., and Wilcoxon, F., *J. Pharmacol. Exptl. Therap.*, **96**, 99(1949).
(16) Ellis, C. H., and Sivertsen, L. N., *Arch. Intern. Pharmacodyn.*, **116**, 17(1958).
(17) Burrell, Z. L., Gittinger, W. C., and Martinez, A. *Am. J. Cardiol.*, **1**, 624(1958).
(18) Goth, A., "Medical Pharmacology," 3rd ed., Mosby, St. Louis, Mo., 1966, p. 371.
(19) Conn, H. L., Jr., and Luchi, R. J., *Am. J. Med.*, **37**, 685(1964).
(20) Ruthen, G. C., *Am. Heart J.*, **70**, 275(1965).
(21) Thyrum, P. T., Conn, H. L., Jr., and Luchi, R. J., *Arch. Intern. Pharmacodyn.*, **151**, 494(1964).
(22) Harrison, D. C., Sprouse, J. H., and Morrow, A. G. *Circulation*, **28**, 486(1963).
(23) Dreifus, L. S., McGarr, T. F., Watanabe, Y., Kline, S. R., Waldman, M., and Likoff, W., *Am. Heart J.*, **65**, 507 (1963).
(24) DiPalma, J. R., *Progr. Cardiovascular Dis.*, **2**, 243 (1960).
(25) Somani, P., and Lun, B. K. B., *J. Pharmacol. Exptl. Therap.*, **147**, 194(1965).

Keyphrases

Antiarrhythmic agents
Procaine amide analogs—synthesis
Pharmacological activity—procaine amide analogs
Atria, guinea pig—antiarrhythmic activity determination
Structure-activity relationship–procaine amide analogs

Linear Nonisothermal Stability Studies

By M. A. ZOGLIO, J. J. WINDHEUSER, R. VATTI, H. V. MAULDING, S. S. KORNBLUM, A. JACOBS, and H. HAMOT

A method for evaluation and utilization of data from linear nonisothermal kinetics has been developed. The studies have yielded energy of activation, reaction rate, and stability predictions from a single experiment. The relatively short length of time needed to complete a study and the comparatively few analytical experiments required present a significant advantage over classical kinetic methods. Large volumes of temperature-controlled space and the need for preliminary screening studies have been eliminated in the method. The advantage of this approach over other nonisothermal kinetic methods lies in the simplicity of equipment required and the ease of analysis of concentration-time (temperature) curves. The hydrolysis of *N*-acetyl-*p*-aminophenol and procainamide hydrochloride has been followed to demonstrate the validity of the theory and the advantages of the method.

THE USE OF stability predictive methods, such as that described in this report and other approaches (1), cannot be expected to replace the formal stability program. The predictive methods should rather be employed in cutting down the number of formulations which are subjected to formal testing by eliminating poor preparations through preformulation as well as formulation screening. The procedures which can be used to predict stability have reached a reasonable degree of sophistication. The recent upsurge of pre-

Received February 13, 1968, from the Pharmacy Research and Development Department, Sandoz Pharmaceuticals, Hanover, NJ 07936
Accepted for publication September 19, 1968.
Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.
The technical assistance of A. Dilatush, D. Murdock, and W. Vincek is gratefully acknowledged.