Anesthetic Properties of Procaine Amide (Pronestyl) and Other Amide Analogues of Local Anesthetic Compounds.* (17638)

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The recent use of N-2-diethylaminoethyl para-amino benzamide HCl in the control of cardiac arrhythmias(1) aroused interest in its anesthetic properties. This substance is the amide analogue of procaine†, its chemical relationship to the parent substance is shown below. No investigation of the anesthetic properties of amine compounds of this type appears to have been previously reported, though, it will be recalled, that anesthetics have been developed which contain one such chain in addition to an alkoxy chain, attached to a quinoline nucleus, as for example Dibucaine (Nupercaine); and several carbanilates having anesthetic properties have previously been investigated(2,3). Preliminary experiments performed on the isolated frog sciatic nerve by techniques previously described by us(4) indicated that procaine amide was relatively inert. It was found, however, that upon intracutaneous or subcutaneous injection in man that this compound caused prompt anesthesia, the duration of which approximated that found for procaine. Accordingly it was decided to investigate these somewhat unusual pharmacological properties of procaine amide in greater detail and to determine its anesthetic index. During the course of these experiments it was decided also to compare the anesthetic properties of Beta-diethylamino-4-ethoxy benzoate (Intracaine) and N- [2-(N-ethyl-N-2-hydroxyethyl)aminoethyl] -p-butoxybenzoate HCl (Compound No. 3) together with their corresponding amide analogues. This paper contains the data obtained from these investigations. The structures of the compounds examined are presented in Table I.

Toxicity. The M.L.D.$_{50}$ for each of the 6 compounds was determined upon mice by the method adopted some years ago in this laboratory(5) and will not again be described. Table II contains the mouse M.L.D.$_{50}$ for the compounds under study. It should be noted that the toxicities of the amides do not bear a constant relationship to the toxicities of the parent substances. It is interesting to note the low toxicity of the amide analogue of Compound No. 3.

Anesthetic Potency. *In vitro* tests upon frogs' sciatic nerves showed that procaine, Intracaine and Compound No. 3 caused prompt interruption of conduction, whereas the corresponding amide analogues of these compounds had little or no effect on the opposite sciatic nerve of the same animal. When tested by intracutaneous and subcutaneous injections in man all the six compounds caused anesthesia and it is noteworthy that the amide analogue of Compound No. 3 was exceedingly rapid in action and caused no initial pain upon injection. Typical data from these experiments are summarized in Table III. *In vivo* tests on rats anesthetized with sodium pentothal showed that these three amide analogues produced little or no block compared with their parent substances when applied to undamaged sciatic nerves.

* This work was supported in part by a grant from E. R. Squibb and Sons.

1. Mark, Berlin, Kayden, Rovenstine, Steele, and Brodie, *Proc. Am. Soc. for Pharm. and Exp. Therap.*, November, 1949.

† This compound is marketed under the trade name of Pronestyl.

2. McIntyre and Sievers, *J. Pharm. and Exp. Therap.*, 1938, v63, 369.

3. Sievers and McIntyre, *J. Pharm. and Exp. Therap.*, 1938, v62, 252.

4. Bennett, Wagner, and McIntyre, *J. Pharm. and Exp. Therap.*, 1942, v75, 125.

5. McIntyre and Sievers, *J. Pharm. and Exp. Therap.*, 1937, v61, 107.

6. Sievers and McIntyre, *J. Pharm. and Exp. Therap.*, 1937, v59, 90.

7. *Pitkin's Conduction Anesthesia*, Lippincott, Philadelphia, 1946, 232.

TABLE I.
Names and Structures of Anesthetic Compounds.

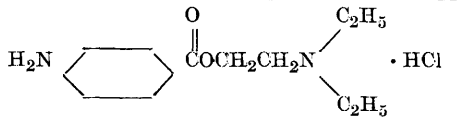
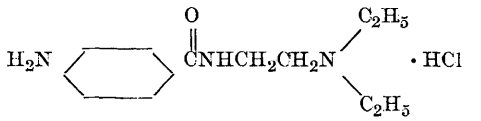
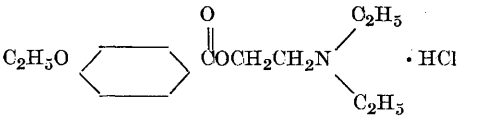
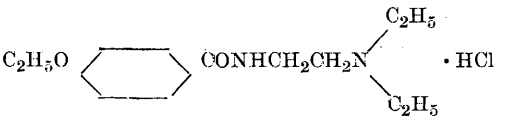
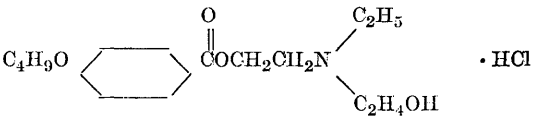
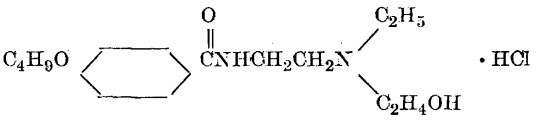
Procaine	
Procaine amide	
Intracaine	
Intracaine amide	
Compound No. 3	
Compound No. 3 amide	

TABLE II.
M.L.D.$_{50}$ and Toxicity Ratios.

Compound	M.L.D.$_{50}$ mouse,* mg/kg	M.L.D. procaine / M.L.D. compound
Procaine HCl	600 ± 20	1.00
Procaine amide HCl	800 ± 30	0.75
Intracaine HCl	430 ± 15	1.40
Intracaine amide HCl	565 ± 40	1.06
	450 ± 20†	1.32
Compound No. 3	320 ± 20	1.87
Compound No. 3 amide	900 ± 100	0.67

* The mice used in these toxicity tests were larger and less resistant to procaine than those generally used in this laboratory.

† The discrepancy is attributed to the high ambient temperature(6).

Because these anesthetics and their amide analogues differed in their actions upon intact sciatic nerves it was decided to determine their partition coefficients between frog Ringer's solution and chloroform. In preliminary experiments equimolar solutions of each of the six compounds were prepared and equal volumes of each were equilibrated with the same volume of chloroform. The Ringer's solution and chloroform were then analysed for nitrogen. However, it was found that solutions of Compound No. 3 in Ringer's solution became turbid at pH higher than 6.6, and it was decided to prepare isotonic saline solutions buffered to pH 6.6 for all the six compounds and to equilibrate with chloro-

TABLE III.
Anesthetic Potency.*

Compound	Frog Sciatic Nerve *in Vitro*: Conc. in frog Ringer's sol., mMol	Time in min. to block	Tests on Man (Intracutaneous): Duration in min.† Range	Mean	Min. effect conc., %	Initial pain in inj.	Erythema	Anesthetic index‡
Procaine HCl	10	5.3	13-18	15	1/40	++	+	1.00
Procaine amide HCl	10	No block in 30 min.	13-21	17	1/40	+++	+++	1.32
	90	" " " " "						
Intracaine HCl	1	9	21-32	25	1/80	+	—	1.42
" amide HCl	1	Slight block in 30 min.	24-36	27	1/80	+	++	1.70
Compound No. 3	0.2	11	28-56	37	1/160	++	++	2.14
" No. 3 amide	0.2	26	31-60	35	1/160	0	0	5.96

* A 0.5% strength in isotonic saline buffer in all experiments.
† A solution of compound No. 3 cannot be buffered to a physiological pH without becoming turbid.
‡ Anesthetic potency of procaine $\frac{\text{M.L.D. compound (7)}}{\text{M.L.D. procaine}}$ / Anesthetic potency of compound

form and again analyse for nitrogen. The results of the two series are presented in Table IV.

Discussion. Examination of the two sets of ratios obtained reveals significant differences in distribution of the compounds between the aqueous and chloroform phases. In both series the amides are less soluble in chloroform than are their parent substances. It seems probable that these differences in solubility are at least in part responsible for the much greater anesthetic activity of the parent substances, upon intact nerve trunks, compared with the relatively weak activity of their amide analogues. Presumably the

TABLE IV.*
Distribution of Anesthetic Hydrochloride Salts Between Water and Chloroform.

Compound (HCl salts)	mMols per liter: Ringer's solution	Chloroform	Ratio†	mMols per liter: Saline buffer pH 6.6	Chloroform	Ratio†
Procaine	9.0	1.70	5.30	8.0	9.9	0.81
" amide	10.0	0.43	23.30	22.0	0.8	24.70
Intracaine	2.9	8.70	0.30	2.4	19.5	0.12
" amide	9.57	0.12	80.00	4.5	16.0	0.28
Compound No. 3	6.90	11.00	0.62	0.0	18.4	0.00
Compound No. 3 amide	6.18	6.18	1.00	1.5	16.0	10.61

* The authors are indebted to Dr. Fred Humoller and Miss Barbara Griswold, of this department, who kindly made the analyses for nitrogen and calculated the data for this table.

† Ratio $= \frac{\text{Millimols in } H_2O}{\text{Millimols in } CHCl_3}$

lipid of the nerve sheath is penetrated relatively slowly by the amides.

Conclusions. 1. The anesthetic potencies of three anesthetics and their amide analogues have been determined and compared.

2. The amide analogues are relatively ineffective as anesthetics upon intact nerve trunks.

3. The partition coefficients between Ringer's solution and chloroform and between buffered isotonic saline of these anesthetics and their amide analogues have been determined.

4. The differences in partition coefficients between these anesthetics and their amide analogues and their pharmacological actions have been discussed.

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Application of Ion Exchange Resins to the Purification of Certain Viruses. (17639)

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The application of ion exchange resins to the separation of closely related chemical substances by adsorption or exchange of ions(1) suggested a possible means of separating viruses from the host tissues upon which they are cultivated. Since virus particles and many host tissue components may be selectively adsorbed by a number of common adsorbents depending upon the pH of the suspending medium, their separation might be effected by passage of an infected tissue suspension through a column of ion exchange resin.

Methods. Amberlite cation exchange resin, XE-64*, between 140 and 180 mesh was used. The resin was placed in a beaker fitted with a mechanical stirrer and converted from the acid to the sodium form with 4% NaOH. After thorough washing with distilled water, the resin was then converted to a mixed sodium-acid form by the gradual addition of concentrated HCl to the resin suspension until the pH of the supernatant was at the desired point between 5.8 and 8.8. Pyrex glass chromatographic columns were then charged with the resin which was supported in the columns on coarse sand and glass wool layered over perforated glass plates. The amount of wet resin employed was twice the volume of virus suspension to be purified. The charged columns were rinsed with distilled water, drained, sterilized in an autoclave and chilled before use.

The viruses used in these experiments were: Japanese encephalitis (Nakayama strain), Eastern equine encephalomyelitis (strain 86), Western equine encephalomyelitis (Rockefeller strain), and rabies virus (CVS strain) obtained from the Virus and Rickettsiae Strain Collection at the Army Medical Department Research and Graduate School. The tissue suspensions were prepared from normal and infected chick embryos and mouse brains homogenized in a Waring Blendor with sufficient phosphate-buffered physiological saline (pH 7.7) to make 5 to 20% suspensions. Following centrifugation at 2000 r.p.m. for 10 minutes to remove coarse tissue particles, aliquots of the cleared suspensions were pipetted into the resin columns. With the aid of vacuum the fluid was pulled through the columns in 15 to 30 minutes. Samples were saved from the original centrifuged suspensions and from the percolates for titration of virus infectivity and for determination of total nitrogen by the micro-Kjeldahl method. The infectivity of preparations was determined by intracerebral titrations using groups of

1. Applezweig, N., *Annals N. Y. Acad. Sci.*, 1948, XLIX, Art. 2, 295.

* Manufactured by Rohm and Haas Co., Philadelphia, Pa.