INFLUENCE OF HALOGEN SUBSTITUTION ON ENZYMATIC HYDROLYSIS

FRANCIS F. FOLDES, M.D., DAVID L. DAVIS, B.S., AND O. J. PLEKSS, M.D.

IN the course of the investigation of the pharmacological properties and the clinical application of 2-chloroprocaine HCl (1), it was observed that this compound is hydrolyzed in human plasma 4 to 5 times faster than procaine HCl (2). Both the low systemic toxicity and the excellent local anesthetic properties of 2-chloroprocaine HCl had been attributed to the substitution of a Cl atom into the procaine HCl molecule. Therefore, it seemed desirable to study the enzymatic hydrolysis and the pharmacological properties of other halogen-substituted benzoic acid derivatives. Several of these benzoic acid derivatives were synthetized under the direction of Dr. L. Reiner.* A preliminary report on the enzymatic hydrolysis (3) and the animal pharmacology (4) of these compounds already has been published, and the clinical results obtained with 2 of the agents will be reported elsewhere. It is the purpose of the authors in this article to report in greater detail on the enzymatic hydrolysis of the halogen substituted benzoic acid derivatives in human plasma and to present briefly some of the toxicity studies carried out with those compounds which proved to be suitable for clinical application.

METHODS

Hydrolysis Studies. The hydrolysis rates of 9 halogen substituted local anesthetic agents, and 3 of their nonhalogenated analogs, were studied in freshly obtained heparinized plasma taken from healthy, young adult volunteers. The hydrolysis rates were measured by a modification of the ultraviolet spectrophotometric method originally suggested by Kalow (5). A 4×10^{-3}M stock solution was made with distilled water of the compounds to be investigated. Before use, the stock solutions were diluted to 1:40 by 0.025M NaCl. This 10^{-4}M solution was used for analysis. Two ml. of the heparinized plasma were also diluted to 10 ml. with a 0.025M NaCl solution. Two ml. of diluted plasma and 2 ml. of the 10^{-4}M solution of the local anesthetic agent to be investigated, previously heated to 37 C., were mixed

This study was supported in part by U.S. Public Health Research Grant No. G-3585. It was conducted in the Department of Anesthesia of the Mercy Hospital and the Section on Anesthesiology of the University of Pittsburgh School of Medicine, and the Pharmaceutical Division of Wallace and Tiernan, Inc., Belleville, New Jersey. The paper was read before the annual meeting of the American Society of Anesthesiologists, Inc., in Cincinnati, on October 25, 1954, and accepted for publication on February 8, 1955.

* Present address: Cancer Research Institute, College of Physicians and Surgeons, Columbia University, New York, New York.

and immediately placed in a cuvette in the sample compartment of a Beckmann DU ultraviolet spectrophotometer. The sample compartment was kept at a constant temperature of 37 ± 0.2 C. by circulating water from a thermostatically controlled bath, with an electric pump, through 2 thermospacers mounted on both sides of the sample compartment. Readings were taken at 1- to 2-min. intervals with one exception at a wavelength of 313 mμ until the hydrolysis was completed. The hydrolysis of 2-diethylaminoethyl-3,4-dichlorobenzoate HCl was studied at a wavelength of 255 mμ. The readings were made against a blank in which the diluted plasma solution was replaced by 2 ml. of 0.025M NaCl. The blank automatically compensated for the nonenzymatic hydrolysis of the various compounds. The hydrolysis was allowed to proceed to completion, indicated by no further change in the optical density of the solution. From the time necessary for 50 per cent hydrolysis, the hydrolysis rates of the various local anesthetic agents were calculated and expressed as μM of substrate hydrolyzed by 1 ml. of plasma in 30 minutes.

Animal Toxicity Studies. The subcutaneous and intravenous LD_{50} of procaine HCl, 2-chloroprocaine HCl, 2-bromoprocaine HCl, chlorocaine-4 HCOOH, and tetracaine HCl were determined in mice weighing about 20 Gm. Groups of 20 to 40 animals were used for each determination. Subcutaneous injections were made under the skin of the back.

The toxicity of procaine HCl, 2-chloroprocaine HCl, chlorocaine-4 HCOOH, and lidocaine HCl on slow intravenous infusion was determined in male albino rabbits weighing 1.8 to 2.3 kg. The infusion was administered through the marginal ear vein at a constant rate of 1 ml. per minute. The concentrations of the solutions were adjusted to the body weight of the animals to permit the administration of the desired number of mg./kg./min. All solutions were maintained at the body temperature of rabbits (38 to 39 C.) during infusion. If death did not occur, the animals were kept under observation for five to six hours. The findings of these experiments were evaluated statistically according to the method of Litchfield and Wilcoxon (6) and expressed as LD_{50} mg./kg./min.

RESULTS

The hydrolysis data obtained with the 13 compounds investigated are summarized in table 1. It is evident from this table that the substitution of a Cl or a Br atom at the 2-position into the procaine HCl molecule (see fig. 1) markedly increased the hydrolysis rate in human plasma. The same was found to be true when Cl was substituted into the tetracaine HCl molecule (see fig. 2) or into the 2-sec.-butyl-aminoethyl-4-aminobenzoate HCl molecule (see fig. 3).

The findings presented also indicate that the hydrolysis of Cl substituted local anesthetic agents is influenced also by the alcohol used

TABLE 1

HYDROLYSIS RATE OF VARIOUS HALOGEN SUBSTITUTED LOCAL ANESTHETIC AGENTS AND SOME OF THEIR NON-HALOGENATED ANALOGS

Compound	Molecular Weight	μM Hydrolyzed by 1 ml. Plasma in 30 minutes	Relative rate* of Hydrolysis
Procaine HCl	272.8	0.79	1.00
2-Chloroprocaine HCl	307.2	3.66	4.63
2-Bromoprocaine HCl	351.7	1.92	2.44
Tetracaine HCl	300.8	0.29	0.36
2-Chlorotetracaine HCl	335.3	1.14	1.44
2-sec.-Butylaminoethyl-4-aminobenzoate HCl	272.8	0.30	0.38
2-sec.-Butylaminoethyl-2-chloro-4-amino-benzoate HCl	307.2	1.56	1.98
2-Isobutylaminoethyl-2-chloro-4-amino-benzoate HCl	307.2	1.83	2.32
2-tert.-Butylaminoethyl-2-chloro-4-amino-benzoate HCl	307.2	0.71	0.90
2-chlorothiocaine HCl	323.3	0.03	0.04
2-Diethylaminoethyl-3,4-dichlorobenzoate HCl	326.7	39.80	50.50
3,5-Dichloroprocaine HCl	341.7	0.20	0.26

* The hydrolysis rate of procaine was chosen unity.

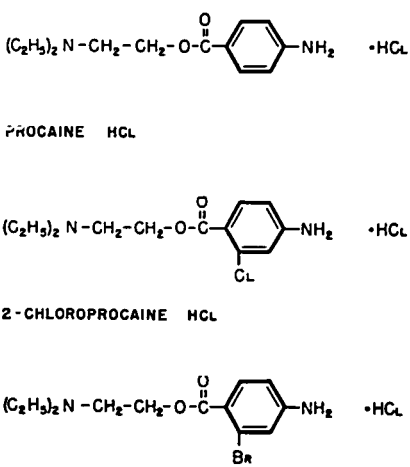
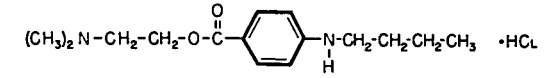
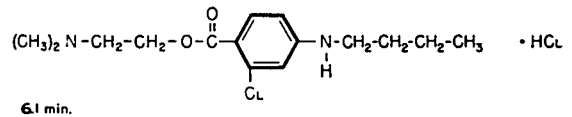


FIG. 1. Structural formulas of procaine HCl, 2-chloroprocaine HCl, and 2-bromoprocaine HCl.



33.0 min.

TETRACAINE HCL



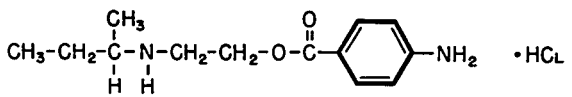
6.1 min.

2-CHLOROTETRACAINE HCL

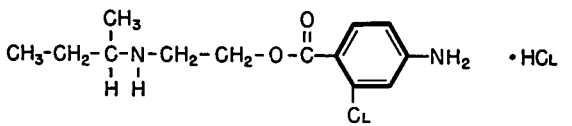
FIG. 2. Structural formulas of tetracaine HCl and 2-chlorotetracaine HCl.

for the esterification of the 2-chloroparamino benzoic acid (see fig. 4). For example, when, instead of the 2-isobutylamino-ethyl alcohol, the 2-tert.-butylaminoethyl alcohol was used for esterification, there was an almost threefold increase in the hydrolysis rate of the resulting compound. Similarly, when, instead of diethylamino-ethyl alcohol, diethylamino-thioethyl alcohol was used (see fig. 4), a sixtyfold decrease in the hydrolysis rate was encountered.

The substitution of 2 Cl atoms into the procaine HCl molecule also

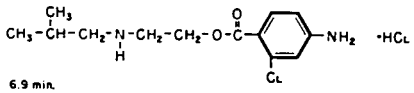


2-SEC.-BUTYLAMINOETHYL-4-AMINOBENZOATE HCL



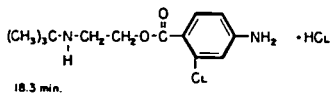
2-SEC.-BUTYLAMINOETHYL-2-CHLORO-4-AMINOBENZOATE HCL

FIG. 3. Structural formulas of 2-sec.-butylaminoethyl-4-aminobenzoate HCl and 2-sec.-butylaminoethyl-2-chloro-4-aminobenzoate HCl.



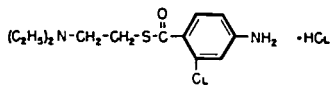
6.9 min.

2-ISOBUTYLAMINOETHYL-2-CHLORO-4-AMINOBENZOATE HCL



18.3 min.

2-TERT.-BUTYLAMINOETHYL-2-CHLORO-4-AMINOBENZOATE HCL

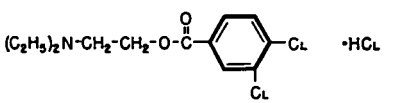


2-CHLOROTHIOCAINE HCL

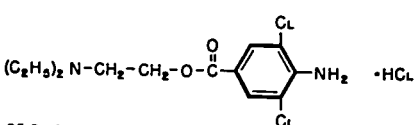
FIG. 4. Structural formulas of 2-isobutylaminoethyl-2-chloro-4 aminobenzoate HCl, tert.-butylaminoethyl-2-chloro-4-aminobenzoate HCl, and 2-chlorothiocaine HCl.

produced a wide variation of the hydrolysis rate in plasma. Depending on the place of substitution, the hydrolysis rate was almost 100 times faster for the 2-diethylaminoethyl-3,4-dichlorobenzoate HCl (see fig. 5) and 6 times slower for the 3,5-dichlorobenzoate HCl.

The results of the subcutaneous toxicity studies are summarized in table 2. The figures of table 2 indicate that the subcutaneous toxicity of the halogen substituted local anesthetic agents, despite their increased potency, is about the same as or less than that of procaine



2-DIETHYLAMINOETHYL-3,4-DICHLOROBENZOATE HCL



65.0 min.

3,5-DICHLOROPROCAINE HCL

FIG. 5. Structural formulas of 2-diethylaminoethyl-3,4-dichlorobenzoate HCl and 3,5-dichloroprocaine HCl.

TABLE 2

TOXICITY OF LOCAL ANESTHETIC AGENTS IN MICE

Compound	Subcutaneous LD_{50} mg./kg.	Relative Rate* of Hydrolysis	Relative† Potency	Safety Index‡ on Subcutaneous Administration
Procaine HCl	615 ± 37	1.0	1.0	1.0
2-Chloroprocaine HCl	1,396 ± 174	4.6	2.0	4.5
2-Bromoprocaine HCl	650 ± 50	2.4	4.0	4.2
Chlorocaine-4 HCOOH	670 ± 56	2.0	4.0	4.3
Tetracaine HCl	48 ± 3	0.4	10.0	0.8

* Compared with procaine HCl in human plasma.

† Compared with procaine HCl in guinea-pig skin wheal tests and in regional nerve blocks in man.

‡ $\frac{LD_{50} \text{ of compound}}{LD_{50} \text{ of procaine HCl}} \times$ Relative potency of compound.

HCl. Their safety index, calculated by multiplying the ratio of their subcutaneous LD_{50} to the subcutaneous LD_{50} of procaine HCl with their relative potency, shows them to be more than 3 times less toxic than procaine HCl or tetracaine HCl.

The greater safety afforded to the halogen substituted local anesthetic agents by their rapid enzymatic hydrolysis rate in plasma is evident also from the results of the experiments carried out with slow intravenous infusion in rabbits. The safety index of the 2 halogen substituted compounds investigated, calculated by multiplying the ratio of their mg./kg./min. LD_{50} to that of procaine HCl by their relative potency, showed that these compounds are relatively less toxic on slow intravenous infusion than procaine HCl (see table 3). Lidocaine HCl, a nonhydrolyzable local anesthetic agent, was found to be absolutely (lowest mg./kg./min. LD_{50}) and relatively (lowest safety index) most toxic.

TABLE 3

TOXICITY OF LOCAL ANESTHETIC AGENTS ON SLOW INTRAVENOUS INFUSION IN RABBITS

Compound	LD_{50} mg./kg./min. and 19/20 Confidence Limits	Relative Toxicity*	Relative Potency†	Safety Index‡
Procaine HCl	2.55 (2.3 to 2.9)	1.0	1.0	1.0
2-Chloroprocaine HCl	2.80 (2.4 to 3.3)	0.9	2.0	2.1
Chlorocaine-4 HCOOH	1.50 (1.4 to 1.7)	1.7	4.0	2.3
Lidocaine HCl	0.80 (0.7 to 0.9)	3.2	2.0	0.6

* Compared with procaine HCl.

† Compared with procaine HCl in guinea-pig skin wheal tests and in regional nerve blocks in man.

‡ $\frac{\text{mg./kg./min. } LD_{50} \text{ of compound}}{\text{mg./kg./min. } LD_{50} \text{ of procaine HCl}} \times$ Relative potency of compound.

DISCUSSION

Since the discovery of procaine by Einhorn in 1905, numerous local anesthetic agents have been synthetized and introduced into clinical use. None of these compounds, however, combined increased potency with decreased systemic toxicity. The unexpected finding, encountered in the investigation of the fate of local anesthetic agents in the human body, that the 2-chloro-analog of procaine HCl was hydrolyzed 4 to 5 times faster than procaine itself (2), focused the attention on a new group of compounds, the halogen substituted local anesthetic agents. It was realized at the onset of these studies that the rapid enzymatic hydrolysis rate of a compound in plasma will make the accumulation of toxic concentrations more difficult, and thereby make the compound more safe for clinical application. It was a fortunate coincidence that the halogen substitution, that increased the hydrolysis rate, in several compounds also increased the potency and the penetrating capacity. It is probable that both the increased rate of the enzymatic hydrolysis and the greater penetrating capacity of the halogen substituted local anesthetic agents are due to their increased polarity.

Despite the serious systemic absorption reactions that may accompany the use of local anesthetic agents, and which not infrequently cause fatalities, relatively little emphasis has been placed in the evaluation of new local anesthetic agents on their systemic toxicity. More often than not, toxic or fatal reactions encountered in clinical practice remain unreported. Instead of safety, other considerations like speed of onset of action, penetrating capacity, and intensity and duration of anesthesia were emphasized in the synthesis of new compounds.

With the exception of their duration of action, the halogen substituted local anesthetic agents so far tested compared very favorably with other clinically used local anesthetic agents. In addition to this, their margin of safety is not only greater than that of the recently introduced potent local anesthetic agents, but also more favorable than that of procaine HCl, the hitherto employed standard of comparison. Although satisfactory duration of action is very important in clinical practice, it cannot be denied that the safety of the patient is an even more important consideration. The duration of action of the halogen substituted local anesthetic agents, depending on the drug selected and the purpose for which they are used, varies from sixty to ninety minutes in epidural block, and from ninety to one hundred and fifty minutes in other forms of regional nerve blocks. This duration is satisfactory for the majority of surgical procedures. When longer duration of action is required, this easily can be obtained with a continuous technique in epidural block, and by repeating the injection in other forms of regional nerve blocks. On occasion, the short duration of action of a local anesthetic agent is an advantage rather than a disadvantage. For example, 2-chloroprocaine HCl in 2 per cent so-

lution can be used, without the admixture of vasopressors, for the production of epidural block on unpremedicated out-patients for the performance of various urological and gynecological procedures. Under such circumstances, the block is fully developed in six to eight minutes, and, after forty-five to sixty minutes, patients are able to go home unaided. Similarly, agents that will rapidly produce intensive nerve block of relatively short duration can be used to advantage in dentistry.

In man, the absolute and relative safety coefficient of the halogen substituted local anesthetic agents is even greater than it is evident from the favorable results of the animal toxicity tests presented. This is due to the fact that the enzymatic hydrolysis rate of ester type local anesthetic agents is 4 to 20 times greater in human plasma than in any other mammalian plasma investigated (8). Consequently, following its clinical administration there is less chance for the accumulation of toxic plasma concentrations than in animal experiments. It should be emphasized once more that, because of the wide species variation of important biochemical mechanisms, animal experiments can only serve as pilot studies in determining the toxicity and the safety of local anesthetic agents, and the final evaluation has to be made on human beings (7).

SUMMARY

1. Halogen substitution in the 2 position of the benzene ring markedly increased the hydrolysis rate of ester type local anesthetic agents.

2. The hydrolysis rate of these halogen substituted local anesthetic agents was influenced also by the structure of the esterifying alcohol and by other changes in the molecule.

3. The substitution of 2 Cl atoms in the benzene ring, depending on the position of the substituent, markedly increased or decreased the hydrolysis rate.

4. The subcutaneous toxicity of the halogen substituted local anesthetic agents in mice was the same as or smaller than that of procaine HCl. Because of their greater potency, however, their safety index was more than 3 times greater than that of procaine HCl.

5. The safety index of the halogen substituted local anesthetic agents on slow intravenous infusion in rabbits was more than twice greater than that of procaine HCl and more than 3 times greater than that of lidocaine HCl.

REFERENCES

1. Foldes, F. F., and McNall, P. G.: 2-Chloroprocaine: New Local Anesthetic Agent, Anesthesiology 13: 287 (May) 1952.

2. Aven, M., and Foldes, F. F.: Chemical Kinetics of Procaine and Chloroprocaine Hydrolysis, Science 114: 206 (Aug.) 1951.

3. Davis, D. L., and Foldes, F. F.: Influence of Halogen Substitution on Enzymatic Hydrolysis of Benzoic Acid Esters with Local Anesthetic Activity, Fed. Proc. 13: 346 (March) 1954.
4. Plekss, O. J., and Reiner, L.: New Substituted Benzoic Acid Ester-Type Local Anesthetics, Fed. Proc. 13: 274 (March) 1954.
5. Kalow, W.: Hydrolysis of Local Anesthetics by Human Serum Cholinesterase, J. Pharm. & Exper. Therap. 104: 122 (Feb.) 1952.
6. Litchfield, J. T., Jr., and Wilcoxon, F.: Simplified Method of Evaluating Dose-Effect Experiment, J. Pharm. & Exper. Therap. 96: 99 (June) 1949.
7. Foldes, F. F., and Rhodes, D. H., Jr.: Role of Plasma Cholinesterase in Anesthesiology, Anes. & Analg. 32: 305 (Sept.–Oct.) 1953.
8. Aven, M. H.; Light, A., and Foldes, F. F.: Hydrolysis of Procaine in Various Mammalian Plasmas, Fed. Proc. 12: 299 (March) 1953.