Metabolism and Measurement of Chloroprocaine, an Ester-Type Local Anesthetic

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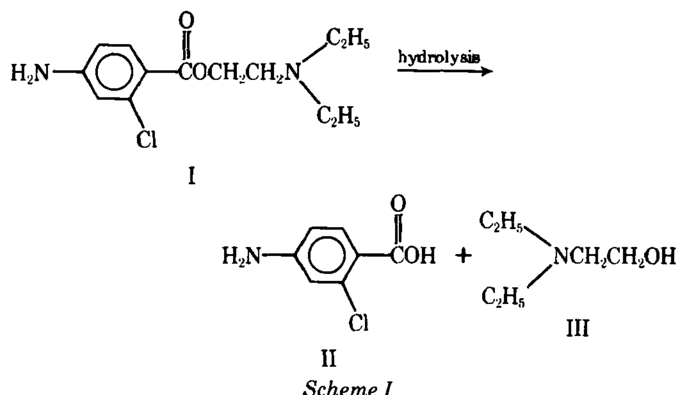
Abstract □ Blood levels of intact chloroprocaine were determined using a GLC procedure capable of detecting as little as 10 ng of chloroprocaine/ml of blood. It was necessary to deactivate plasma cholinesterases in collected samples by adding echothiophate iodide to blood sampling tubes. An extraction procedure, followed by a TLC separation and spectrodensitometric assay, was developed for measurement of the metabolite 2-chloro-4-aminobenzoic acid in plasma and urine. With these procedures, data were obtained showing that chloroprocaine is hydrolyzed rapidly by plasma cholinesterases. No unchanged drug could be detected in any blood samples obtained from volunteers who received chloroprocaine by intravenous infusion or from obstetric patients who had epidural anesthesia during labor and delivery. Blood levels of 2-chloro-4-aminobenzoic acid rose promptly with the administration of chloroprocaine and declined rapidly after drug administration. Most of the metabolite was recovered in urine.

Keyphrases □ Chloroprocaine—GLC analysis in plasma and urine, metabolism and elimination in humans □ GLC—analysis, chloroprocaine in plasma and urine □ Metabolism—chloroprocaine in humans □ Elimination—chloroprocaine in humans □ Anesthetics, local—chloroprocaine, GLC analysis in plasma and urine, metabolism and elimination in humans

Structurally, the local anesthetics commonly used to relieve pain during surgical and obstetrical procedures are of two main types: amides and esters. The amide-type agents tend to be longer acting, because they are more stable toward hydrolysis and are not rapidly metabolized. As a consequence, when administered repeatedly to obstetric patients during labor, these agents may accumulate in high or even toxic concentrations in the maternal and fetal blood. Neonatal depression, fetal bradycardia, methemoglobinemia, and even fetal deaths have been attributed to the toxicity of these drugs (1–4). In contrast, chloroprocaine[1] (I), an ester-type local anesthetic, is hydrolyzed rapidly by the maternal and fetal plasma pseudocholinesterase into two relatively nontoxic metabolites, 2-chloro-4-aminobenzoic acid (II) and 2-diethylaminoethanol[2] (III) (Scheme I) (6, 7).

The objective of this study was to ascertain whether the apparent low toxicity (8) of I can be attributed to its rapid metabolism resulting in low levels of intact drug in the maternal and/or fetal blood. Therefore, it was necessary to develop sensitive and selective procedures capable of measuring the intact agent and a metabolite, II, in biological fluids. The approach described involves: (*a*) collection of blood samples over echothiophate, which completely inhibits plasma cholinesterase activity; (*b*) separation of the plasma fraction to facilitate handling and shipping of the samples; (*c*) extraction of either I or II into an organic solvent; and (*d*) measurement of the drug or metabolite by GLC or by the emitted fluorescence of a treated TLC plate.



Scheme I

EXPERIMENTAL

GLC Determination of Chloroprocaine (I)—The following modified procedure of Somogyi (9) was employed to precipitate protein. A 5-ml aliquot of 1.8% (w/v) $Ba(OH)_2 \cdot 8H_2O$ was added to 2 ml of plasma in a 50-ml centrifuge tube, and the mixture was shaken for 45 sec. This time was long enough to ensure good contact and complete protein precipitation, yet short enough to minimize losses due to alkaline hydrolysis[3]. A 5-ml aliquot of 2% (w/v) $ZnSO_4 \cdot 7H_2O$ was added to the sample. Compound I dissolved in this neutral solution was stable throughout the remainder of the procedure.

The precipitated protein was separated from the liquid by centrifugation, and the supernate was decanted into a 50-ml graduated tube. The precipitate was washed twice, by repeating the above procedure, using 2 ml of 1.8% $Ba(OH)_2$ and 2 ml of 2% $ZnSO_4$. These washes were added to the initial liquor, the combined volume was read, and the entire sample was transferred to a 30-ml separator. The pH was adjusted to 8.5 with 2% (w/v) sodium bicarbonate.

The aqueous phase then was extracted with methylene chloride[4] three times; 5 ml was used for the initial two extracts, and 2 ml was used for the final one. The extracts were placed into a 15-ml centrifuge tube, and the combined volume was recorded. At this time, the internal standard [100 µl of 0.001% (w/v) procaine in methylene chloride] was added to the extract. To act as a scavenger and to minimize drug losses that may occur by adsorption on glass surfaces, 1 ml of 0.02% (w/v) triethanolamine in methylene chloride was then added.

The methylene chloride was removed under a gentle stream of nitrogen at 50°. The walls of the tube were washed down with 2 ml of methylene chloride, and again the solvent was removed. The residue was reconstituted with 100 µl of methylene chloride, and a 5-µl sample was injected into a biomedical gas chromatograph[5]. The instrument, operated isothermally at 220°, was equipped with a flame-ionization detector and a 1.2-m long × 3-mm i.d. glass column packed with 10% SE-30 on 80–100-mesh Chromosorb W. The injection port temperature was 230°, and the detector temperature was 270°. The flow rates were 40 ml/min at 40

[1] Nesacaine, Pennwalt Corp., Rochester, N.Y.
[2] A recently published paper by Raj *et al.* (5) described a spectrophotometric approach to measuring I levels directly in plasma. Efforts to duplicate the procedure were not successful, primarily because of sample opacity and/or component interference.

[3] The ester linkage of I is susceptible to hydrolysis, either through base catalysis or by plasma cholinesterases. For that reason, the contact time at the elevated pH must be minimized and the enzymic hydrolysis must be inhibited completely to obtain reliable data.
[4] Pesticide grade, Fisher Scientific.
[5] Hewlett-Packard model 7610 A.

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Table I—Recovery of Chloroprocaine Added to Whole Blood

I Added, ng/ml of blood	Amount Recovered, ng/ml	Percent Recovery
100	97	97
100	100	100
250	252	101
250	234	94
625	628	100
625	656	105
1250	1242	99
1250	1311	105
2500	2524	101
2500	2483	99
Mean		100
SD		3

psig for the carrier gas (helium), 35 ml/min for hydrogen, and 300 ml/min for air.

A typical chromatogram is shown in Fig. 1. Under these conditions, the practical lower limit of detection for I was ≃ 10 ng/ml of plasma.

Quantitative TLC Estimation of 2-Chloro-4-aminobenzoic Acid (II)—A volume of fluid (for samples taken during anesthetic procedures, usually 5 ml of plasma or 2 ml of urine) containing a minimum of 50 ng of II was made acidic (pH 1–3) by addition of 1 ml of a saturated ammonium chloride solution and a few drops of concentrated hydrochloric acid. The metabolite was extracted into an equivalent volume of ethyl acetate as the mixture was agitated on a rotary mixer[6] for 10 min.

The two phases were separated by centrifugation and spotted on a F-254 silica gel (0.25 mm thickness) TLC plate[7]. The individual components were separated by acetic acid–methanol–chloroform (2:18:80 v/v). A pilot run may be required so that the volume spotted and/or the concentration of the ethyl acetate solution can be adjusted to obtain a spot containing 50–200 ng of II, the linear range of response by the spectrodensitometer[8].

The plate was developed to within 2 cm of the top and dried. It was then exposed to acetic acid vapors and sprayed with 0.01% fluorescamine[9]. The amount of fluorescence developed at the incident wavelength of 370 nm was quantitated with the spectrodensitometer. The intensity of the emitted green fluorescence (wavelength around 450 nm) approximated a linear relationship with concentration over a range of 50–200 ng/spot.

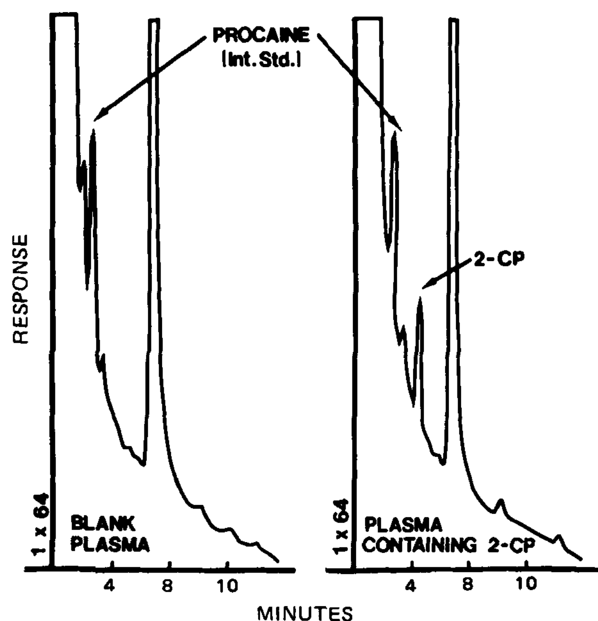

Figure 1—*Typical chromatograms of extracts of human plasma (2-CP = chloroprocaine).*

[6] Lab Quake, Labindustries, Berkeley, Calif.
[7] E. Merck, Brinkmann Instruments, Westbury, N.Y.
[8] Model SC3000, Schoeffel Instruments, Westwood, N.J.
[9] Fluram, Roche Diagnostics, Nutley, N.J.

Table II—Recovery of 2-Chloro-4-aminobenzoic Acid Added to Plasma or Urine

Plasma		Urine	
Added, μg/ml	Percent Recovery	Added, μg/ml	Percent Recovery
5.0	112	100.4	94
5.0	102	100.4	95
5.0	106	100.4	94
10.1	97	100.4	90
10.1	97	376.4	88
10.1	98	376.4	91
		376.4	91
		376.4	92
Mean	102		92
SD	6		2

Most of II is excreted in the urine as conjugates, which were hydrolyzed by treating with an equivalent volume of 1 *N* NaOH at 100° for 20 hr. If acid hydrolysis is employed in lieu of the base treatment, decarboxylation, resulting in the formation of 2-chloroaniline, will occur.

***In Vitro* Estimation of Hydrolysis Rate**—Protein precipitation was initially used to diminish the background interference caused by substances endogenous to plasma; subsequently, it was employed to terminate enzymatic hydrolysis in preparation for the acquisition of kinetic data.

Blood samples from obstetrical patients and cord blood from their newborns, as well as blood from healthy volunteers, were collected in heparinized blood sampling tubes[10].

While the sample was agitated on a vortex mixer, a known amount of I was added rapidly as an aqueous solution (20 μl of approximately 250 ng of I/μl of water) to 2 ml of blood or plasma at 23°. At a preselected time, the enzymatic hydrolysis was terminated by protein precipitation and the residual II was extracted and determined as described. Since the reaction is very rapid, it was necessary for the analyst to act expeditiously. Pilot measurements indicated that a half-life of 10–30 sec can be expected for the intact drug in normal plasma. With this criterion, sampling times of 2, 10, 20, and 60 sec were adequate.

***In Vivo* Studies**—*Sampling Procedures*—It would not have been practical to terminate enzymatic action *via* protein precipitation while blood was being drawn from patients or volunteers. Therefore, a program was initiated to investigate cholinesterase inhibitors. Echothiophate iodide rapidly and completely inhibited the cholinesterase activity of blood, and it was used to modify sample tubes according to the following procedure.

Commercially available, heparinized, 10-ml blood sampling tubes[10] were modified by the addition of 50 mg of echothiophate iodide[11] (aqueous solution, 100 μl of 0.5 g/ml) to each tube. The tubes were then stoppered, and a vacuum of 200 torr was reestablished. To preserve the integrity of the inhibitor, the tubes were refrigerated until used.

Intravenous Infusion Studies—Seven healthy volunteers, four males and three nonpregnant females, each received 250 mg of I (2% concentration) by constant rate intravenous infusion. These seven volunteers were divided into two groups. The first group of three received the infusion over 60 min. Since no side effects were observed, the infusion rate was then increased to 250 mg/30 min for the second group of four.

A total of 12 blood samples per individual was drawn into the modified tubes from the antecubital vein in the contralateral arm at preselected times during the infusion and up to 30 min after it was terminated. Urine samples were collected 60 and 90 min after the beginning of infusion, and their volumes were measured and recorded. Plasma fractions were separated promptly by centrifugation and frozen along with the urine samples until analyzed. Attempts to measure I were made on all samples. Determination of II levels were made only in the second group of four volunteers.

Obstetric Epidural Anesthesia—One patient in labor received continuous epidural anesthesia over 3 hr preceding delivery. Anesthesia was induced and maintained with 2% I, for a total dose of 740 mg. Maternal venous blood samples were drawn into echothiophate-containing tubes at 10, 20, 30, and 60 min after the induction of anesthesia. Another maternal sample was obtained at delivery, along with fetal samples from the umbilical artery and vein. These samples were analyzed for I only.

Two other patients receiving continuous epidural anesthesia during labor were studied. At birth, maternal venous, umbilical venous, and umbilical arterial samples were collected for determination of I and II.

[10] Vacutainer, Becton-Dickinson, Rutherford, N.J.
[11] Ayerst Laboratories, New York, N.Y.

Table III—Half-Life (Seconds) of Chloroprocaine in Maternal and Umbilical Cord Blood

Subject	Maternal	Umbilical Cord
1	13	23
2	15	38
3	19	37
4	21	45
5	22	58
6	27	47
7	29	50
Mean	21[a]	43[a]

[a] Paired t test; $p < 0.001$.

Table IV—Half-Life (Seconds) of Chloroprocaine in Samples of Whole Blood Obtained from Male and Female Controls

	Males	Females
	14	24
	22	27
	25	31
	22	21
	20	22
		26
Mean	21[a]	25[a]
SD	4	4

[a] Pooled t test; $0.10 < p < 0.20$.

RESULTS AND DISCUSSION

GLC Determination of I—The feasibility of using GLC techniques was demonstrated for the determination of I in biological fluids. An initial protein precipitation was required to reduce background interference from endogenous plasma constituents. While Table I provides data showing recovery of a known addition of I (100–2500 ng/ml) from fresh prepoisoned human blood, a practical detection limit of 10 ng/ml could be attained[12].

Quantitative TLC Estimation of II—In all of the subjects studied, I was hydrolyzed so rapidly that the intact drug could not be detected. Therefore, the drug disposition was followed indirectly by determining levels of one of its primary metabolites, II, by the previously described extraction and quantitative TLC procedure.

To evaluate the method, known amounts of II were added to blood and urine samples. According to the described procedures, these samples were acidified and extracted with ethyl acetate and then the metabolite content of the extract was determined by the quantitative TLC procedure. The data in Table II show good agreement between the amounts added and those recovered. The practical detectable limit was about 50 ng of II/spot.

While the procedure is applicable to whole blood measurement, it is more convenient to use the plasma fraction. Therefore, the possibility of preferential distribution between the erythrocyte fraction and plasma must be considered. A known amount of II was added to each of four whole blood samples, which were then incubated at 37° for 1 hr. The plasma from two of the samples was then separated. The II content of these fractions was compared to that in the remaining two whole blood samples, and no significant differences in concentration could be observed. Because of the relative ease of handling, plasma samples were used for subsequent measurements.

***In Vitro* Estimation of Hydrolysis Rate**—To gain insight into the detoxification mechanisms of I, its hydrolysis rates were determined in maternal and fetal plasma samples (Table III), as well as in samples obtained from healthy male and nonpregnant female volunteers (Table IV). These determinations were performed at room temperature (23°). The rates of *in vivo* metabolism at 37° should be faster.

Since the cholinesterase activity of the neonate's plasma appears to be related to that of the mother, and since the sampling of the male and female population was independent, the statistical comparison of maternal and cord plasma was made by a paired t test while the comparison between male and female plasma was made according to a pooled t test. The observed mean plasma half-life of I was shorter in males than in females; however, this difference was not significant. In contrast, the hydrolysis of I was much faster in maternal than in fetal plasma; the differences of observed mean values of approximately 21 and 43 sec, respectively, were highly significant.

***In Vivo* Studies**—*Sampling Procedures*—To prevent artifactual drug hydrolysis in the collected samples, it was necessary to develop an effective and convenient technique of completely inhibiting cholinesterase in the sampling tube. Several potential inhibitors were compared relative to protein precipitation (Table V). To be acceptable, the material had to meet the following requirements: (*a*) complete and rapid inhibition of cholinesterase activity, (*b*) lack of interference in the final GLC measurements, and (c) convenience in clinical usage. Only echothiophate at the 2-mg/ml level possessed the required characteristics.

To confirm further the general acceptability of echothiophate as an inhibitor, a known quantity of I (3.8 μg/tube) was added to heparinized blood sampling tubes, previously modified with echothiophate. These tubes were used for blood collection from four healthy volunteers. The samples were then analyzed for residual I content (Table VI). From the 23 measurements, a mean recovery of 75% (*SD* = 4) was obtained, indicating that approximately 25% of the drug was hydrolyzed prior to complete enzyme deactivation. Obviously, a finite time lapse is unavoidable before the required contact between blood cholinesterases and echothiophate can be established. This interval is apparently sufficient to allow hydrolysis of 25% of the added drug. Despite this problem, the data demonstrate that echothiophate is an excellent agent for terminating enzymatic hydrolysis of I and that its use provides reliable blood samples for subsequent assay.

[12] Unpublished data.

Table V—Plasma Cholinesterase Inhibition by Various Substances

Inhibitor	Concentration of Inhibitor in Blood, mg/ml	Percent Recovery of Drug after 30 min
Protein precipitation	—	100
Sodium arsenite	5–50	10–50
Sodium fluoride	5–15	50
Dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide[a]	0.5–5	50
Sodium fluoride plus dimethylcarbamate	0.5–15	50
Echothiophate iodide[b]	0.5	80
	1.0	90
	2.0	100

[a] Hoffmann-La Roche Inc. [b] Ayerst Laboratories.

Table VI—Recovery of Chloroprocaine Showing the Effectiveness of Echothiophate-Modified Tubes

Subject	n[a]	Percent Recovery Mean	*SD*
1	6	74	4
2	5	79	6
3	6	73	3
4	6	74	3

[a] Three samples per subject in duplicate (one tube for Subject 2 was lost).

Table VII—2-Chloro-4-aminobenzoic Acid Content (Micrograms per Milliliter) of Plasma in Subjects Receiving 250 mg of Chloroprocaine/30 min by Intravenous Infusion

Minutes	Subject 1	Subject 2	Subject 3	Subject 4
2	Trace	Trace	0.2	0.71
5	0.2	0.9	1.2	2.7
10	2.2	2.2	2.6	2.4
15	3.0	2.6	3.9	2.9
30	—[a]	3.5	4.3	3.6
Infusion terminated				
35	2.6	—[a]	2.2	1.5
40	2.6	0.7	2.0	1.0
45	2.2	0.6	1.8	0.7
60	0.8	Nil	0.6	0.6

[a] Lost sample.

Table VIII—Recovery of 2-Chloro-4-aminobenzoic Acid and Its Conjugates Emitted in the Urine 60 and 90 min after the Beginning of an Intravenous Infusion of 250 mg of Chloroprocaine/30 min

	60 min				90 min				Total			
	Free		Conjugate		Free		Conjugate		Free		Conjugate	
Subject	mg	% dose	mg	% dose	mg	% dose	mg	% dose	mg	% dose	mg	% dose
1	10.3	6.6	13.9	8.9	0.5	0.3	20.2	13.0	10.8	6.8	34.1	21.9
2	2.0	1.3	14.5	9.3	8.3	5.3	74.8	47.9	10.3	6.6	89.3	57.1
3	3.9	2.5	43.1	25.1	Trace	—	17.6	11.3	3.9	2.5	60.7	38.8
4	13.0	8.3	50.9	32.6	0.4	0.4	36.6	23.3	13.4	8.6	87.5	56.0

Intravenous Infusion Studies—Compound I could not be detected in any sample from volunteers receiving an intravenous infusion. These results suggest either that essentially all of the drug disappeared from the circulation at a rate equal to or greater than the rate at which it was being infused or that plasma cholinesterases had not been deactivated completely by the inhibitor during sample collection. If the latter were the case, hydrolysis would continue until the protein precipitation procedure was carried out.

The extremely rapid hydrolysis rate shown by *in vitro* experiments suggested that the first alternative was more likely. Nonetheless, to test the second alternative, known quantities of I were added to aliquots of blood samples obtained in the abovementioned protocol. After the samples stood for several minutes, they were assayed for residual compound. No loss of drug occurred, indicating that echothiophate had, indeed, deactivated the plasma cholinesterases.

Initial measurements for I were negative. No intact drug could be observed in any of the seven subjects. The distribution and excretion of I were then determined indirectly by measuring II concentrations in blood and urine of the group of four who received the drug over 30 min (Table VII). The acid metabolic fragment, II, was present. Its concentrations increased with time, rapidly at first, and then tended to plateau. After the infusion was terminated, the blood levels of II declined at rapid rates, either because of further metabolism or through an excretory process.

As shown in Table VIII, urinary elimination was the primary means of removing this metabolite. The listed results were derived from urine samples collected from the same four volunteers at 60 and 90 min from the beginning of infusion. As much as 65% of the administered dose was found in the urine after 90 min. Most of the II was excreted as a conjugate of which no definite identification has been made, although β-glucuronidase and sulfatase had no effect upon the release of II. In analogy to unsubstituted benzoic acid and salicylic acid, the glycine conjugate could be among the more likely products (10). Regardless of its composition, the proportion of the conjugate to the free metabolite increased with time.

Table IX—Levels (Micrograms per Milliliter of Plasma) of Anesthetic and Its Metabolite in Maternal and Umbilical Cord Plasma of Two Patients Who Received Approximately 800 mg of Chloroprocaine for Obstetric Epidural Anesthesia

	I		II	
Sample	Patient 1	Patient 2	Patient 1	Patient 2
Umbilical artery	Nil	Nil	0.03	0.09
Umbilical vein	Nil	Nil	0.07	0.45
Maternal vein	Nil	Nil	1.97	2.78

Obstetric Epidural Anesthesia—No intact I could be found in the maternal or umbilical cord blood samples following obstetric epidural anesthesia. Rapid metabolism within the maternal circulation probably prevented the drug from reaching the fetus. Measurable quantities of II were found in all samples (Table IX). They were higher in the umbilical venous than umbilical arterial blood, suggesting the transfer of the metabolite into the fetus. However, the low ratios of fetal to maternal levels of II indicate that the placental transfer of this compound was very limited.

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