Plasma Levels of 2-Chloroprocaine in Obstetric Patients and Their Neonates after Epidural Anesthesia

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The purpose of this study was to determine maternal and fetal plasma levels of 2-chloroprocaine following epidural anesthesia during labor, and to examine its metabolism by plasma cholinesterase to 2-chloroaminobenzoic acid (CABA). The study population included 35 normal patients whose infants were delivered vaginally or by repeat cesarean section, and their infants. Gas chromatographic techniques were used to determine concentrations of 2-chloroprocaine and CABA in plasma; spectrophotometric techniques were used to determine plasma cholinesterase activity. In maternal plasma 2-chloroprocaine was detectable for at least 5–10 min after each dose; mean levels at delivery were 25 ± 80 and 51 ± 13 ng/ml for patients having cesarean section and vaginal delivery, respectively. In contrast, CABA was detectable throughout labor. In cord blood plasma, 2-chloroprocaine was detectable in half of the cord-vein and arterial samples analyzed; the highest mean concentration was 17 ng/ml in samples from vaginally delivered infants. CABA was detectable in three quarters of the cord blood samples. Plasma cholinesterase activity was found to be low in both mothers and neonates, and further decreased following anesthesia in both groups. In maternal plasma, cholinesterase activity was 42 per cent less per ml plasma compared with that for nonpregnant controls, and 80 per cent less following anesthesia compared with that measured prior to anesthesia. In cord blood plasma, cholinesterase activity was 22 per cent less than that found in samples from nonpregnant women and 70 per cent less in infants whose mothers received 2-chloroprocaine than in control infants. These data suggest that the decreased activities of maternal and neonatal cholinesterases at term are adequate to hydrolyze most, but not all, of the plasma 2-chloroprocaine following epidural anesthesia during labor.

(Key words: Anesthesia, obstetric. Anesthetic techniques: epidural; peridural; lumbar. Anesthetics, local: chloroprocaine. Metabolites: 2-chloroaminobenzoic acid. Enzymes: pseudocholinesterase. Measurement techniques: gas chromatography.)

2-CHLOROPROCaine is rapidly becoming the local anesthetic of choice for obstetric anesthesia.1 There are several reasons for this. First, it is rapidly hydrolyzed by plasma cholinesterases and therefore is unlikely to reach toxic levels in plasma.2 Second, the products of hydrolysis—2-chloroaminobenzoic acid (CABA) and 2-diethylaminomethyl—are both pharmacologically inactive.2 Finally, neither maternal nor fetal plasma levels of unchanged 2-chloroprocaine have ever been detected. Thus, placental transfer of the drug has not been documented.2,3 The rapid and theoretically complete hydrolysis of 2-chloroprocaine by plasma cholinesterases apparently explains why unchanged 2-chloroprocaine has not previously been measurable in either maternal or fetal plasma.

The purpose of this investigation was to use sensitive gas chromatographic techniques to confirm or refute previous reports that 2-chloroprocaine cannot be measured in maternal and fetal plasma, and to examine its metabolism by plasma cholinesterases following epidural anesthesia during labor.

Methods and Materials

Maternal or cord plasma samples from 33 pregnant patients at term were included in this study; the infants of 14 patients were delivered vaginally and those of 19 patients by cesarean section. Informed consent was obtained and epidural anesthesia with 2-chloroprocaine was judged to be clinically appropriate by the attending physician. Patients with hepatic or renal disease, and those with histories of drug addiction, were excluded from the study. Patients with mild complications of pregnancy such as gestational diabetes, mild anemia, mild preeclampsia, or premature rupture of the membranes were included so long as epidural anesthesia was appropriate. The characteristics of the study population were: age 24 ± 5 years; race 19 black, 14 white; parity 8 nulliparous, 25 multiparous. All infants were healthy term neonates with Apgar scores of 7 or more at 1 min of life.

Chloroprocaine, 2 or 3 per cent, without ephinephrine was administered through an epidural catheter by the anesthesiologist as needed for pain relief. The mean number of doses needed by individual patients was 3 ± 2, and mean numbers of milligrams needed were 468 ± 284 and 948 ± 347 for vaginal delivery and cesarean section, respectively. The mean time intervals between the initial injection of 2-chloroprocaine and delivery were 122 ± 83 min for vaginal delivery and 50 ± 25 min for cesarean section; the

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intervals between the final injection and delivery were 19 ± 33 and 23 ± 15 min, respectively.

Maternal blood samples (5 ml) were collected from an indwelling cannula in a superficial vein on the dorsum of the hand 1, 3, 5, 7, 10, 20, 30, 60, and every 30 min after the epidural injection or until a repeat dose or delivery occurred. Thirty maternal samples were obtained at delivery coincident with clamping of the infant's cord. Duplicate maternal samples were obtained at delivery from 11 patients; 27 duplicate cord-vein and 21 cord-artery samples were obtained from doubly clamped sections of umbilical cord. All samples were immediately put into heparinized Vacutainers® and placed on ice. Samples to be analyzed for 2-chloroprocaine and CABA levels were collected in Vacutainers containing 0.3 ml of a cholinesterase inhibitor, echthiopate iodide, 0.2 g/ml (Ayerst Laboratories); samples analyzed for cholinesterase activity were obtained in Vacutainers without the inhibitor. The plasma was removed following centrifugation and frozen until assayed for drug levels or cholinesterase activity.

For comparison, blood was also obtained from ten nonpregnant healthy volunteers of childbearing age and was analyzed for plasma cholinesterase activity. All volunteers were nurses or other hospital personnel, and informed consent was obtained in each case. In addition, cord blood samples were obtained from doubly clamped cords from 11 healthy term infants whose mothers did not receive 2-chloroprocaine during labor or delivery (eight mothers received lidocaine, two received meperidine, one received no medication); these samples were also analyzed for cholinesterase activity.

To be sure that no patient with atypical cholinesterase activity was being studied, plasma cholinesterase activity and the dibucaine number were determined for each pregnant patient using the spectrophotometric method of Kalow and Genest.® Plasma samples from ten nonpregnant volunteers and cord-vein plasma samples from infants whose mothers did not receive 2-chloroprocaine were studied in an identical manner. Cholinesterase activity was determined in all of the maternal preinjection and cord-vein samples and also in 11 of the maternal samples collected at delivery.

Plasma samples were analyzed separately for 2-chloroprocaine and CABA using modifications of the gas chromatographic technique of Mather and Tucker.® Details of the analyses are presented in the Addendum.

**Results**

Low levels of unchanged 2-chloroprocaine were measured in plasma samples from patients receiving...
TABLE 1. Maternal and Cord Plasma Concentrations (Mean ± SD) of 2-Chloroprocaine and CABA at Delivery

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>2-Chloroprocaine (mg/ml)</th>
<th>CABA (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>18</td>
<td>28 ± 80 (0–335)*</td>
<td>8 ± 12 (1–46)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>12</td>
<td>51 ± 13 (0–470)</td>
<td>2 ± 3 (0–8)</td>
</tr>
<tr>
<td>Cord vein plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>19</td>
<td>4 ± 7 (0–25)</td>
<td>7 ± 8 (0–25)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>12</td>
<td>17 ± 32 (0–92)</td>
<td>0.6 ± 0.6 (0–1.3)</td>
</tr>
<tr>
<td>Cord artery plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>16</td>
<td>5 ± 13 (0–52)</td>
<td>5 ± 10 (0–32)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>8</td>
<td>9 ± 18 (0–52)</td>
<td>1 ± 1 (0–4)</td>
</tr>
</tbody>
</table>

* Range.

At delivery, 2-chloroprocaine was detectable in many maternal and cord blood plasma samples (table 1): unchanged 2-chloroprocaine was detectable in 16 of 30 maternal samples, in 15 of 30 cord-vein samples, and in nine of 24 cord-artery samples analyzed. Mean maternal levels at delivery were less than 51 ng/ml and cord levels were less than 17 ng/ml. In 11 cases 2-chloroprocaine was detectable in both maternal and cord-vein samples and the calculated fetal:maternal ratio was 0.92 ± 0.63 (mean ± SD). CABA was detectable in all but one maternal sample and in 36 of 46 cord-vein and cord-artery samples.

The dibucaine numbers of all 23 pregnant patients and ten controls were normal (82 ± 11). However, prior to exposure to 2-chloroprocaine, enzymatic activity in pregnant patients in labor was 42 per cent less per ml plasma than that in the nonpregnant female volunteers (table 2). In addition, enzymatic activity measurable at delivery following exposure to 2-chloroprocaine was 80 per cent less than that measured prior to anesthesia (table 3).

Plasma cholinesterase activity in cord-vein blood was found to be 70 per cent less for infants whose mothers received 2-chloroprocaine than for infants whose mothers received other medication (table 3). Also, the levels of plasma cholinesterase activity in cord-vein blood from the control infants were found to be 25 per cent lower (P < 0.05) than those found for nonpregnant women (table 2), but not significantly different from the lowered levels found in premedication samples from obstetric patients (tables 2 and 3).

**Discussion**

Analyzing unchanged 2-chloroprocaine in obstetric patients and their neonates requires very sensitive analytical techniques. The procedure we used was successful because it was maximized for sensitivity in several ways: First, the use of a nitrogen phosphorus gas chromatographic detector allowed detection of 2-chloroprocaine at a much lower level than the flame ionization detector used by either O'Brien et al. or Smith et al. Second, the extraction procedure was designed to minimize in-vitro loss of the drug. This was accomplished by not using a harsh alkaline protein precipitation step, using a very mild base for alkaline extraction, adding an internal standard before extraction, eliminating an evaporation step, concentrating the sample in a very small final volume, and utilizing a two-step back-extraction technique. Finally, our sampling times (1, 3, 5, . . . min) after each dose allowed us to see the maximum amount of drug prior to its hydrolysis by plasma esterases.

It is well known that cholinesterase levels are de-

![Table 2. Plasma Cholinesterase Activities (Mean ± SD) in Obstetric Patients and Nonpregnant Controls](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931470/)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Enzymatic Activity (μM AChE/ml/hr, 37°C)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant controls</td>
<td>10</td>
<td>215 ± 69 (146–386)*</td>
<td>—</td>
</tr>
<tr>
<td>Obstetrical patients</td>
<td>23</td>
<td>125 ± 56 (62–288)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Range.

![Table 3. The Effects of Exposure to 2-Chloroprocaine on Maternal and Cord-vein Plasma Cholinesterase Activities](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931470/)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Enzymatic Activity (μM AChE/ml/hr, 37°C)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-injection</td>
<td>11</td>
<td>138 ± 63 (92–288)*</td>
<td>—</td>
</tr>
<tr>
<td>Delivery</td>
<td>11</td>
<td>28 ± 36 (0–96)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cord vein plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other medication</td>
<td>11</td>
<td>161 ± 22 (124–189)</td>
<td>—</td>
</tr>
<tr>
<td>2-Chloroprocaine</td>
<td>25</td>
<td>49 ± 65 (0–254)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Range.
cent reductions, respectively, in cholinesterase activity in healthy newborns compared with nonpregnant adults. Lowered enzymatic activity in newborns was also observed by Abderhalden and Lehmann et al. and indirectly by other investigators, who reported that the half lives of compounds metabolized by plasma cholinesterase (procaine and 2-chloroprocaine) are twice as long in newborns as in adults. Our finding of significantly less enzymatic activity in control infants (whose mothers did not receive 2-chloroprocaine) than in nonpregnant women supports these earlier investigations.

The results of this study convincingly demonstrate that 2-chloroprocaine crosses the placenta: Unchanged 2-chloroprocaine was measurable in half of the cord-vein samples. However, additional evidence for placental transfer, even in the absence of detectable 2-chloroprocaine in cord blood, is the inhibition of cord-vein cholinesterase activity observed in infants whose mothers received 2-chloroprocaine. The 70 per cent decrease in enzymatic activity found in these infants is of the same magnitude as the 80 per cent decrease present before and after medication in the maternal plasma. These data suggest that in many cases the already low level of fetal enzymatic activity increased per ml of plasma during pregnancy. This decrease has been reported to range from 16 to 31 per cent of nonpregnant levels, depending upon the methods used to measure enzymatic activity. Using the method of Kalow and Genest, we also observed a significant decrease in enzymatic activity in pregnant patients compared with nonpregnant controls. However, we report a decrease of 42 per cent, which is higher than that previously reported. This discrepancy is probably due to differences in analytic techniques or the populations studied. Nevertheless, our results support those of earlier investigators.

The results of the present study also demonstrated that the lowered levels of plasma cholinesterases present at term are adequate to hydrolyze most, but not all, of the 2-chloroprocaine diffusing into the blood following epidural anesthesia. This conclusion is warranted for two reasons: First, obvious disappearance profiles of plasma 2-chloroprocaine could be obtained following single large doses of 2-chloroprocaine for cesarean section, as well as following multiple doses for vaginal delivery. Second, the large reduction in measurable enzymatic activity before and after anesthesia with 2-chloroprocaine suggests that the maternal esterases are being inhibited by the presence of excess substrate.

It has been reported previously that plasma cholinesterase activity is depressed at birth: Zsigmond and Downs and McCance et al. observed 50 and 40 per cent decreases, respectively, in cord-vein samples. Our data suggest that this decrease is not due to a reduction in enzymatic activity resulting from the metabolic processes during the birth process, but rather is due to a decrease in the amount of enzyme present.
is being further inhibited by excess substrate in the same manner as is the maternal enzymatic activity.

It is not known whether there is any biologic significance to the placental transfer of low levels of 2-chloroprocaine or the greatly inhibited cord-vein cholinesterase activity in the normal infant. The levels of unchanged 2-chloroprocaine are extremely low and probably short-lived; there is no known biologic role for the plasma cholinesterases other than the metabolism of foreign compounds. Thus, even though unchanged 2-chloroprocaine can reach the fetus, its low toxicity and inactive metabolites appear to justify its preference over amide-linked drugs for obstetric anesthesia.

Addendum

Gas chromatography was used to determine the levels of 2-chloroprocaine in plasma using a modification of the technique for ester-linked compounds suggested by Mather and Tucker. Standard curves were made using plasma inhibited by echorhiolate iodide and procaine§ (0.5 µg, 50 µl) as the internal standard. Pure crystalline chloroprocaine was obtained from the Pennwalt Corporation, Rochester, New York. Sodium carbonate, 2 m, 100 µl, was used for the first alkaline extraction and 2 m, 200 µl, was used for the second alkaline extraction. The final extraction of 2-chloroprocaine was into chloroform, 25 µl (MCB-chromatography).

A Hewlett Packard 5840-A gas chromatograph equipped with a nitrogen phosphorus detector was used to quantitate 2-chloroprocaine. The gas chromatographic conditions suggested by Mather and Tucker were modified to include the following short temperature program: temperature 1–225 °C, time 1–2.5 min; rate 30 degrees/min, temperature 2–255 °C, time 2–2.3 min. An attenuation change at 3 min was also necessary for the lower concentrations. Using this program, retention times (Rf) are 1.98 min for procaine and 3.75 min for chloroprocaine (fig. 3). The chloroprocaine peak is separate from that of a contaminant which often elutes at 4 min. The standard curve obtained using peak height ratios allows quantitation of chloroprocaine to 3 ng/ml.

A second aliquot of each plasma sample was analyzed for CABA using p-aminobenzoic acid (PABA)** as the internal standard. PABA, 1 mg, 100 µl, was added to plasma, 1 ml, which was then acidified to pH 2. Methylene chloride, 3 ml, was added and the mixture rotated on a vortex mixer for 2 min. The samples were then centrifuged at 2,500 rpm for 2 min or longer if necessary because of emulsification. The upper aqueous layer was discarded. The methylene chloride extract was then dried over anhydrous NaSO₄ crystals for 20 min. Following centrifugation, the dry methylene chloride was transferred to a 3-ml Reacti-vial†† and evaporated to dryness under nitrogen at 80 °C in a dry heating block. Next, N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), † † 100 µl, containing 1 per cent trimethylchlorosilane (TMCS), † † and 5 µl of dry pyridine, † † were added under nitrogen for derivatization. The final mixture was sealed with a Te-f bond cap † † † † and heated for 45 min at 80 °C.

The column and flow conditions for gas chromatographic analysis of CABA are the same as those for chloroprocaine. The temperature program is: temperature 1–190 C, time 1–2.5 min; rate 15 degrees/min; temperature 2–250 C; time 2–3 min. The Rₚ of PABA is 2.43 min and that for CABA, 4.23 min (fig. 4). The attenuation is changed at 3 and 6 min. A large contaminant peak at 7.8 min necessitates the long program. The standard curve obtained allows quantitation to 0.3 µg/ml plasma.

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References


§ Applied Sciences, State College, Pennsylvania.
¶ Pennwalt Corporation, Rochester, New York.