

## Contrasting Effects of Etomidate and Propylene Glycol Upon Enflurane Metabolism and Adrenal Steroidogenesis in Fischer 344 Rats

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This study was designed to investigate the effects of etomidate and its solubilizing agent (propylene glycol) upon enflurane metabolism and adrenal steroidogenesis in Fischer 344 rats. A central venous catheter was placed using pentobarbital anesthesia, and rats were randomized to one of four groups for treatment several days later. Group 1 animals received normal saline, 3 ml/kg, given via the central venous catheter. The other three groups were administered equivalent volumes of either: crystalline etomidate (group 2), 0.4 mg/ml, in saline and 1.1% ethanol; propylene glycol (group 3), 7%, in saline; or etomidate (group 4), 0.4 mg/ml in saline with 7% propylene glycol. In the first part of this study, after an intravenous bolus of one of these four solutions, animals were immediately placed in a 200-liter chamber and received 1 h of 2% enflurane. Serum and urine were assayed for inorganic fluoride ( $F^-$ ) before and after anesthesia. Two hours after enflurane anesthesia, groups 1 and 2 had the highest mean peak serum  $F^-$  concentrations (13.2 and 13.5  $\mu M$ , respectively). Groups 3 and 4 had significantly lower mean peak serum  $F^-$  concentrations (4.7 and 4.5  $\mu M$ , respectively). In the second part of this study, additional animals were randomized into four groups and received the same intravenous medications as above. Thirty minutes later, they received an intravenous bolus of ACTH. Blood samples were drawn and serum aldosterone levels were measured. Animals in groups 1 and 3 had significantly greater increases in peak serum aldosterone levels 30 minutes after ACTH (peak levels: 0.80 and 0.77 ng/ml, respectively) than animals in groups 2 and 4 (peak levels: 0.60 and 0.58 ng/ml, respectively). This study demonstrated that propylene glycol inhibited the *in vivo* metabolism of enflurane. In contrast, propylene glycol did not appear to play any role in the inhibition of adrenal steroidogenesis associated with etomidate. (Key words: Anesthetics, intravenous: Amidate®; etomidate. Anesthetics, volatile: enflurane. Biotransformation: fluorometabolites; inhibition. Ions: fluoride. Metabolism: adrenal steroidogenesis; ACTH; aldosterone. Rats: Fischer 344. Vehicle: propylene glycol.)

ETOMIDATE IS A short-acting intravenous anesthetic agent, formulated with 35% (V/V) propylene glycol (Amidate®). Its use has been associated with inhibition of adrenal steroidogenesis.<sup>1-5</sup> This side effect has been

attributed to inhibition of two adrenal cytochrome P-450-dependent mitochondrial enzymes, cholesterol side-chain cleavage enzyme and 11- $\beta$ -hydroxylase.<sup>6</sup> Etomidate does not appear to have any inhibitory effects upon the microsomal cytochrome P-450-dependent enzymes of adrenal steroid synthesis. In contrast, however, etomidate has been shown to have inhibitory effects upon hepatic microsomal cytochrome P-450-dependent metabolism *in vitro*.<sup>7</sup>

In a clinical study, we observed essentially no metabolism of enflurane to inorganic fluoride ( $F^-$ ) in one patient who received Amidate®, as well as methohexital, for induction. This observation implied that etomidate inhibited hepatic drug metabolizing enzymes in humans.

This animal study was therefore designed to examine the effect of Amidate®, etomidate, and its solubilizing agent, propylene glycol, upon the *in vivo* metabolism of enflurane, as measured by the production of inorganic fluoride ( $F^-$ ) following enflurane anesthesia. The results of this study led us to a second question. This was, did propylene glycol play a role in the observed inhibitory effects of etomidate upon adrenal steroidogenesis *in vivo*, as measured by the serum aldosterone response to an ACTH stimulation test?

### Methods and Materials

#### ANIMALS

Seventy, 6-month-old, male Fischer 344 rats<sup>¶</sup> were bedded on hardwood bedding,\*\* four to a cage, and quarantined for a week prior to experimentation. Room temperature was maintained at  $21 \pm 1^\circ C$  and artificial light was provided from 6 A.M. to 7 P.M. each day. Rat chow<sup>††</sup> containing 21 mg/kg of  $F^-$  and tap water containing 1 ppm of  $F^-$  (52.6  $\mu M$ ) were allowed *ad libitum* throughout the experiment, which lasted 14 days.

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## ENFLURANE METABOLISM STUDY

Forty rats were anesthetized with pentobarbital (60 mg/kg, i.p.) for insertion of a central venous catheter *via* the right external jugular vein. After recovery from anesthesia, they were housed individually in metabolic cages until the end of the experiment. The central venous catheters were flushed daily with a minimal volume of 0.9% saline with 30 units of heparin/ml to maintain catheter patency to injection. Two consecutive days of 24-h urine collections for urine volumes and  $F^-$  excretions were made commencing four days prior to enflurane anesthesia. At the end of the urine collections, a blood sample for analysis of serum  $F^-$  was drawn from the tail.

**Treatment Groups and Enflurane Exposure.** Thirty-two of the above 40 rats were selected for exposure to enflurane based on evaluations of catheter patency (necessary for drug injection), body weights, and urine volumes (indicators of general health following surgery). Rats were ranked by weight and randomly assigned (within weight ranges) to one of the treatment groups, such that groups averaged  $323 \pm 15$  g. Each rat received 3 ml/kg body weight of one of the following solutions *via* the central venous catheter. The four groups were: 1) saline (9 mg/ml sodium chloride); 2) crystalline free base etomidate, 0.4 mg/ml, in 1.1% ethanol (for solubilization) in saline; 3) propylene glycol, 7% (V/V) in saline; and 4) Amidate® diluted in saline to 0.4 mg/ml etomidate and 7% propylene glycol. This dose of etomidate was selected from pilot studies in other rats because it produced brief anesthesia (<10 min) equivalent to that seen in humans after an induction dose of etomidate (0.3 mg/kg). The low solubility of etomidate in saline, even with ethanol, determined the total volume of injectate for all groups. The solution for group 4 was diluted such that etomidate concentrations were equivalent to that of group 2. Group 3 injectate was prepared with the same final concentration of propylene glycol as group 4.

Ten rats were assigned to each of groups 1 and 2 because of the greater variability in enflurane defluorination seen in control groups in pilot studies performed in other rats. Six rats were assigned to each of groups 3 and 4. From pilot work, the use of a saline/ethanol control group (*i.e.*, no etomidate) in addition to a saline control group was not indicated.

Following injection, rats were exposed to 2% enflurane for 1 h in a 200-liter plexiglass anesthetic chamber. Enflurane was vaporized in an Ohio Medical vaporizer with compressed air and  $O_2$ . Enflurane concentrations were continuously monitored by a Puritan Bennett Anesthesia Agent Monitor® and confirmed at 2–5-min intervals by gas chromatography with a F.I.D. The chamber  $O_2$  concentration was monitored with an In-

strumentation Laboratories #402  $O_2$  monitor, and was maintained at 25%. Rectal temperatures of representative rats were monitored with a Yellow Springs Telethermometer®, and body temperatures were maintained normothermic with heated water mattresses under the chamber floor.

Blood samples for analysis of serum  $F^-$  were drawn from the tail at 0, 2, and 4 h following enflurane anesthesia. A final blood sample was taken following  $CO_2$  administration and decapitation 24 h postanesthesia. Two 24-h urine samples were collected prior to enflurane anesthesia; a single 24-h urine sample was collected following anesthesia. Serum and urinary samples were analyzed for  $F^-$  with an Orion® Ion Specific  $F^-$  electrode and Corning® Ion Meter #135.<sup>8</sup> Urinary volumes were measured and  $F^-$  excretions were calculated.

At the end of the study, positioning of the central venous catheters was confirmed by necropsy in group 1 and 3 rats. Necropsies were not performed in rats of group 2 and 4; all animals in these groups manifested characteristic signs of etomidate anesthesia immediately upon injection, and, thus, these catheters were assumed to be correctly positioned.

## STEROID INHIBITION STUDY

The 30 rats in this part of the study were similarly anesthetized with pentobarbital for insertion of a right external jugular central venous catheter. After anesthesia, they were placed in individual cages. The central venous catheters were flushed daily with minimal volumes of a 25% dextrose solution containing heparin, 500 units/ml. Pilot studies demonstrated that this hygroscopic mixture would maintain a higher incidence of catheter patency and allow free drawback of blood for several days after cannulation in more rats than our previously utilized regimen with 30 units of heparin/ml in saline. Thus, sampling of blood could occur at frequent intervals, while causing the least amount of stress-induced disturbance to the animal's hormonal status.

Three days after catheter placement, the 20 rats with catheters patent to both injection of solution and drawback of blood were randomly assigned by weight to the same four treatment groups (saline, etomidate, propylene glycol, and Amidate®). A control sample of blood for aldosterone measurement was drawn and the appropriate solution was then injected intravenously. Thirty minutes later, 0.2  $\mu$ g of ACTH (Cortrosyn®, Organon Pharmaceuticals) was injected intravenously. This dose of ACTH was selected from pilot studies in other rats, because it stimulated a measurable aldosterone release, but a release which peaked close to the upper limit of accuracy of our aldosterone assay. Higher

ACTH doses in pilot studies both exceeded the upper limit of the assay and prolonged the period of elevated aldosterone. Blood samples for aldosterone measurement were then drawn 30, 60, and 90 min later. Serum aldosterone was measured by a radioimmunoassay method (Aldosterone RIA, Abbott Laboratories). Samples from 30 and 60 min were measured in duplicate.

### STATISTICS

Serum  $F^-$  and aldosterone concentrations and urinary  $F^-$  excretion data each were analyzed by repeated measures analysis of variance (ANOVA) with treatment as the grouping factor and times of collection as the repeated measure. A square-root transformation was used for urinary  $F^-$  data to produce homogenous variances. Where significant  $F$  ratios were observed for data, *post hoc* analysis with ANOVA was performed among groups at specific sample times. For analyses exhibiting a significant  $F$  ratio at these specific times, intergroup differences were isolated by Newman-Keuls contrast test. Paired  $t$  test was used to identify within-group differences in urinary  $F^-$  excretions.

Where significant  $F$  ratios were observed in serum aldosterone concentrations in response to ACTH, an ANOVA of the difference score between serum aldosterone levels pre- and 30 min post-ACTH administration (equivalent to an analysis of the within-subjects effects from a repeated measures ANOVA with two measurements on each subject) was performed. Significant effects were then isolated to specific groups by Newman-Keuls contrast test.  $P < 0.05$  was considered significant.

### Results

#### ENFLURANE METABOLISM STUDY

The rise in serum  $F^-$  after enflurane anesthesia (fig. 1) peaked within 2 h in rats receiving saline (group 1; 13.2  $\mu$ M) or etomidate in saline/ethanol (group 2; 13.5  $\mu$ M). Serum  $F^-$  concentrations in rats receiving propylene glycol (groups 3 and 4) peaked at the end of anesthesia (4.7 and 4.5  $\mu$ M, respectively). Serum  $F^-$  levels in groups 3 and 4 were significantly less than those in groups 1 and 2 at 0, 2, and 4 h following enflurane exposure ( $P < 0.01$ ).

Urinary  $F^-$  excretions post-enflurane exposure in groups 1 and 2 were significantly increased from pre-anesthetic levels ( $P < 0.05$ ; table 1). Groups 1 and 2 excreted significantly more  $F^-$  than group 3 ( $P < 0.05$ ). Propylene glycol-treated rats (group 3) showed no significant increase in urinary  $F^-$  excretion following enflurane exposure.

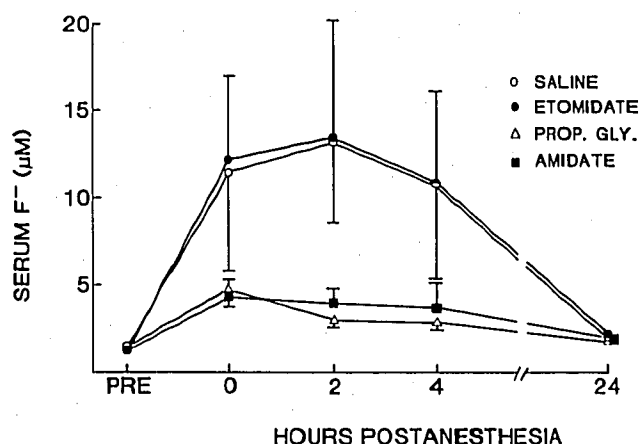


FIG. 1. Serum  $F^-$  concentrations (mean  $\pm$  SD) of rats prior to and following 1 h of 2% enflurane. After anesthetic exposure (*i.e.*, 0, 2, and 4 h), serum  $F^-$  concentrations in groups 1 (saline) and 2 (etomidate) were significantly higher than in groups 3 (propylene glycol) and 4 (Amidate®);  $P < 0.01$  for all comparisons. No differences among groups were present prior to or 24 h following exposure.

#### STEROID INHIBITION STUDY

After ACTH administration, serum aldosterone levels rose and peaked at 30 min (table 2). At 60 min, mean values were not different among groups (range 0.52–0.57 ng/ml); serum aldosterone levels had returned to control by 90 min. Elevations in serum aldosterone in animals receiving etomidate or Amidate® were significantly less than those receiving saline or propylene glycol ( $P < 0.05$ ).

#### Discussion

This study demonstrated that metabolism of enflurane, as measured by changes in serum  $F^-$ , was reduced following the administration of Amidate®. Surprisingly, a similar effect was also observed in animals receiving

TABLE 1. Urinary  $F^-$  Excretions (Mean  $\pm$  SD)

| Treatment Group            | n  | umoles $F^-$ /24 h |                |                |
|----------------------------|----|--------------------|----------------|----------------|
|                            |    | Pre Enflurane      |                | Post Enflurane |
|                            |    | Sampling Day 1     | Sampling Day 2 | Day 1          |
| Group 1 (saline)           | 10 | 2.7 $\pm$ 1.0      | 2.5 $\pm$ 0.8  | 9.7 $\pm$ 3.9* |
| Group 2 (etomidate)        | 10 | 2.6 $\pm$ 1.1      | 2.1 $\pm$ 1.0  | 9.7 $\pm$ 6.3† |
| Group 3 (propylene glycol) | 6  | 2.9 $\pm$ 0.4      | 3.1 $\pm$ 0.8  | 4.0 $\pm$ 2.4  |
| Group 4 (Amidate®)         | 6  | 2.8 $\pm$ 0.6      | 2.6 $\pm$ 0.8  | 4.7 $\pm$ 2.3  |

\* Saline vs. propylene glycol,  $P < 0.05$ .

† Etomidate vs. propylene glycol,  $P < 0.05$ .

TABLE 2. Serum Aldosterone Values (Mean  $\pm$  SD)  
from ACTH Stimulation Test

|                               | n | ng Aldosterone/ml Serum |                     |                             |
|-------------------------------|---|-------------------------|---------------------|-----------------------------|
|                               |   | Pre<br>ACTH             | 30 min Post<br>ACTH | Difference<br>Pre to 30 min |
| Group 1<br>(saline)           | 5 | .41 $\pm$ .17           | .80 $\pm$ .13       | .38 $\pm$ .24*              |
| Group 2<br>(etomidate)        | 5 | .52 $\pm$ .14           | .60 $\pm$ .09       | .07 $\pm$ .09               |
| Group 3<br>(propylene glycol) | 5 | .29 $\pm$ .08           | .77 $\pm$ .17       | .48 $\pm$ .11†              |
| Group 4<br>(Amidate®)         | 5 | .40 $\pm$ .17           | .58 $\pm$ .12       | .18 $\pm$ .06               |

\* Saline vs. etomidate or Amidate®,  $P < 0.05$ .† Propylene glycol vs. etomidate or Amidate®,  $P < 0.05$ .

propylene glycol alone, the solubilizing agent for etomidate, but not in animals receiving etomidate alone. Thus, the *in vivo* inhibitory effect of Amidate® upon enflurane metabolism can be attributed to propylene glycol, and not to etomidate. Propylene glycol alone did not appear to have an effect upon adrenal responsiveness, as measured by the serum aldosterone response to ACTH administration. Furthermore, the effects of etomidate on adrenal steroidogenesis were not enhanced by propylene glycol.

The clinical use of etomidate has been questioned because of its ability to inhibit adrenal steroidogenesis and the possibility of resultant side effects.<sup>9</sup> The mechanism for this interaction has been studied extensively and attributed to inhibition of two cytochrome P-450-dependent mitochondrial enzymes.<sup>6</sup> It might be expected that etomidate would possess inhibitory effects on other enzyme systems. Indeed, Horai *et al.*<sup>7</sup> demonstrated that etomidate reversibly inhibited the activity of several hepatic microsomal enzymes *in vitro*. Inhibition of the cytochrome P-450-dependent enzyme system is potentially significant because this system is responsible for metabolism of a great number of drugs, including the fluorinated volatile anesthetic agents.

Our isolated observation that Amidate® inhibited metabolism of enflurane in a patient appeared to support this possibility. However, in this *in vivo* rat study, it was propylene glycol, not etomidate, which produced significant effects upon the metabolism of enflurane. The Fischer 344 rat has repeatedly been demonstrated to be a useful model of human anesthetic biotransformation,<sup>10-12</sup> and, thus, it is likely that the propylene glycol in Amidate® prevented metabolism of enflurane to F<sup>-</sup> in our patient.

The second part of this study showed that inhibition of adrenal steroidogenesis by etomidate was not influenced by either of the two solvents (propylene glycol or saline/ethanol) studied. In rats, corticosterone is

both the active glucocorticoid and the precursor to aldosterone.<sup>13</sup> Thus, changes in plasma aldosterone are an indirect reflection of the effect of ACTH upon glucocorticoid formation. At the time this study was performed, measurement of serum corticosterone was very expensive, technically complicated, and time consuming compared to assay of aldosterone. Therefore, serum aldosterone was assessed as our measure of adrenal responsiveness to ACTH.

The exact mechanism for our observations of decreased enflurane metabolism is not clear. Nelson *et al.*<sup>14</sup> reported that the concurrent administration of propylene glycol did not affect antipyrine clearance in humans. Their study, however, could not exclude the possibility that propylene glycol might interfere with cytochrome P-450-mediated drug metabolism. There have been no reports of a direct toxic effect of propylene glycol upon drug metabolizing enzyme systems. Repeated administrations of drug solutions containing propylene glycol, however, have been associated with hyperosmolality and CNS depression.<sup>15,16</sup> Lactic acidosis has also been reported, apparently resulting from propylene glycol metabolism to lactic acid *via* intermediates.<sup>17</sup>

*In vitro* animal studies have shown that prolonged oral and intraperitoneal administration of propylene glycol produced either no change in or variable enhancement of hepatic cytochrome P-450-dependent metabolism of several substrates.<sup>18,19</sup> In an *in vivo* study with rats, 3 days of twice daily intraperitoneal injections of 4 ml/kg of propylene glycol (doses considerably in excess of our single intravenous injection of 3 ml/kg of a 7% propylene glycol solution) increased sleeping times following hexobarbital and zoxazolamine administration.<sup>18</sup> These observed effects might indicate an inhibition of drug metabolism, but, alternatively, they could suggest an additive effect of the CNS depressant effects of hexobarbital or zoxazolamine and those of propylene glycol.

The mechanism for the observed inhibitory effect of propylene glycol on enflurane metabolism was not addressed in the present study. However, it is clear from this study and previous reports that propylene glycol cannot be considered a totally innocuous drug solubilizing agent.

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