Pharmacokinetics and Pharmacodynamics of Methylecgonidinine, a Crack Cocaine Pyrolyzate

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ABSTRACT

Methylecgonidinine is formed from cocaine base when smoked and has been identified in biological fluids of crack smokers. Ecgonidin, a metabolite of methylecgonidinine formed via esterase activity, also has been identified in similar samples collected from crack smokers. Methylecgonidinine and ecgonidin can be used as biomarkers to differentiate smoking from cocaine use via other routes of administration. We determined the pharmacokinetic properties of methylecgonidinine and ecgonidin in sheep after intravenous administration of methylecgonidinine at doses of 3.0, 5.6, and 10.0 mg/kg using gas chromatography-mass spectrometric assays. Methylecgonidinine clears quickly from blood with a half-life of 18 to 21 min, whereas ecgonidin has a longer half-life of 94 to 137 min. Because ecgonidin clears more slowly, it may be a more effective biomarker of cocaine smoking. The cardiovascular stimulant effects of cocaine contrast with reported in vitro muscarinic agonist effects of methylecgonidinine, decreasing contractility and stimulating nitric oxide production in cardiac cells and tissues. To test the hypothesis that methylecgonidinine produces cardiovascular effects in vivo consistent with muscarinic agonism, methylecgonidinine was administered to sheep intravenously (0.1–3.0 mg/kg) while monitoring heart rate and blood pressure. Significant hypotension and tachycardia occurred in all three sheep. Two of the three sheep demonstrated mild bradycardia 3 to 5 min after methylecgonidinine injection. Intravenous pretreatment with atropine methyl bromide (15 μg/kg) antagonized methylecgonidinine-induced hypotension in all three sheep, supporting the hypothesis that methylecgonidinine acts as a muscarinic agonist in vivo.

Heating cocaine base (smoking crack) produces the pyrolyzate methylecgonidinine (anhydroecgonine methylester). Because methylecgonidinine is produced via thermal conversion from cocaine, it can be used as a marker to differentiate between smoking and cocaine use via other routes of administration. In forensic cases, methylecgonidinine has been detected in urine (Jacob et al., 1990; Cone et al., 1994; Paul et al., 1999; Riley et al., 2001; Shimomura et al., 2001), blood/plasma (Jenkins and Goldberger, 1997; Toennes et al., 1999; Shimomura et al., 2001; Fandino et al., 2002; Toennes et al., 2003), saliva (Kintz et al., 1997), perspiration (Kintz et al., 1997), hair (Kintz et al., 1995, 1997), and brain and liver (Shimomura et al., 2001) of crack smokers. Although methylecgonidinine has been identified in numerous matrices, the number of blood or plasma samples in which it has been detected numbers less than 50 in the literature. The maximum methylecgonidinine blood or plasma concentration accurately reported in the literature is 110 ng/ml (Toennes et al., 2003), whereas the maximal urinary concentration is 6340 ng/ml (Riley et al., 2001). We hypothesized that methylecgonidinine is rapidly cleared from the central compartment accounting for low blood, plasma, and tissue concentrations. Therefore, we administered methylecgonidinine intravenously to sheep and examined its clearance from blood.

Methylecgonidinine is metabolized to ecgonidin (anhydroecgonine) by butyrylcholine esterase activity, accounting for the presence of ecgonidin in urine and postmortem brain and liver (Paul et al., 1999; Shimomura et al., 2001; Fandino et al., 2002) (Fig. 1A). These investigators and others hypothesized that ecgonidin is the principal metabolite of methylecgonidinine, undergoing demethylation analogous to cocaine demethylation forming benzoylecgonine (Jacob et al., 1990; Paul et al., 1999; Shimomura et al., 2001). Urinary ecgonidin concentrations were greater than methylecgonidinine in forensic samples, suggesting that ecgonidin is a major metabolite, having a longer half-life than methylecgonidinine and should be a longer persisting indicator of crack smoking than methylecgonidinine (Paul et al., 1999). In our present studies, we analyzed blood samples for methylecgonidinine and ecgonidin concentrations to determine their pharmacokinetics, testing the hypothesis that ecgonidin has a longer half-life than methylecgonidinine.

ABBREVIATIONS: AMB, atropine methyl bromide; MAP, mean arterial pressure.
ecgonidine and ecgonidine formation from methylecgonidine. B, structures of laboratory synthesized ethylecgonidine and N-ethyl-N-norecgonidine, internal standards for methylecgonidine and ecgonidine plasma assays, respectively.

Intravenous methylecgonidine administration produces cardiovascular effects that differ from cocaine. Erzouki et al. (1995) reported that 1.0 mg of cocaine administered intravenously to anesthetized rabbits increased mean blood pressure and peak heart rate; however, 3.0 mg of methylecgonidine also administered intravenously decreased mean blood pressure and peak heart rate (Erzouki et al., 1995). These responses to methylecgonidine mimic muscarinic agonism (Brown and Taylor, 1996). Several in vitro studies have demonstrated that methylecgonidine acts as M₂ muscarinic agonist (Huang et al., 1997; Woolf et al., 1997; Yang et al., 2001, 2002). Therefore, we wanted to test the hypothesis that the pharmacodynamics of methylecgonidine are consistent with muscarinic agonism in awake sheep. In addition to methylecgonidine’s cardiovascular effects, we examined whether methylecgonidine could be antagonized by atropine methyl bromide (AMB), a nonspecific muscarinic antagonist with limited access to the central nervous system.

Materials and Methods

Reagents and Assay Standards

Methylecgonidine and ethylecgonidine fumarate, ecgonidine, and N-ethyl-N-norecgonidine hydrochloride (Fig. 1B) were synthesized as reported previously (Scheidweiler et al., 2000). Atropine methyl bromide was purchased from Sigma-Aldrich (St. Louis, MO). Methacholine chloride was purchased from Metapharm, Inc. (Brantford, ON, Canada). CleanScreen ZSDAU020 columns (United Chemical Technologies, Bristol, PA) were used for solid phase extraction. All solvents were reagent or high-performance liquid chromatography grade (Fisher Scientific Co., Fair Lawn, NJ).

Methylecgonidine and Ecgonidine Assay

Calibrators for methylecgonidine and ecgonidine were prepared in blank sheep plasma at the following concentrations: 30, 50, 100, 250, and 500 ng/ml. Assay controls [55, 300, and 2500 ng/ml (3000 ng/ml ecgonidine)] were prepared in blank sheep plasma. Methylecgonidine and ecgonidine were extracted from plasma samples via solid phase extraction using CleanScreen columns after methanol precipitation of protein and analyzed by gas chromatography-mass spectrometry (Scheidweiler et al., 2000).

Gas Chromatography-Mass Spectrometry Analysis

The gas chromatography-mass spectrometry-selected ion monitoring settings for methylecgonidine and ecgonidine assays were detailed previously (Scheidweiler et al., 2000). Methylecgonidine and ecgonidine recovery was greater than 75%. Assay variation was evaluated with methylecgonidine or ecgonidine controls; coefficients of variation were less than 10% for 55, 300, and 2500 ng/ml (3000 ng/ml for ecgonidine) controls. The methylecgonidine assay linearity was from 20 to 2500 ng/ml with a limit of detection of 10 ng/ml. Ecgonidine linearity ranged from 30 to 3000 ng/ml with a limit of detection of 10 ng/ml. Concentrated study samples were diluted to within assay linearity using distilled water before extraction.

Experimental Design

Two separate experiments were conducted for this study, an initial pharmacokinetic experiment and a follow-up pharmacodynamic experiment. Our institutional Animal Care and Use Committee approved these animal studies. Animals were housed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Pharmacokinetic Study. Nonpregnant female sheep of mixed breed were purchased from a local vendor and prepared with indwelling femoral catheters (1.5 mm i.d. by 2.3 mm o.d. Tygon microbore tubing, formulation S-54-HL; Norton Performance Plastics, Akron, OH) while under general anesthesia using aseptic technique. At least 1 week was allowed for recovery after surgery. Methylecgonidine fumarate was dissolved in sterile saline for injections (part 366471; BD Biosciences, Franklin Lakes, NJ) were used for collecting blood samples (2–3 ml). Sodium fluoride tubes limit plasma esterase activity and aid stability of methylecgonidine after sample collection (Scheidweiler et al., 2000). Venous blood sampling covered from 5 min before methylecgonidine injection until at least 55 min after methylecgonidine administration. Samples were collected every 30 s for first 5 min, every minute until 10 min, every 2 min until 20 min, and every 5 min until 55 min after administration. Samples were placed on ice immediately, centrifuged at 1000 × g for 20 min, and plasma was collected and stored at –80°C until analysis.

Noncompartmental Pharmacokinetic Calculations. The venous line, used for both drug administration and blood collection was flushed with 5 ml of saline after drug administration. Inspection of residuals after nonlinear regression fitting of the entire methylecgonidine elimination profiles revealed that data points collected 2 min after drug administration were outliers, showing as falsely elevated methylecgonidine plasma levels. Thus, these samples were not included in pharmacokinetic analysis. The terminal linear phase of elimination was fit on the log-transformed plasma concentrations to the model \(C(t) = C_0 e^{-kt}\), where \(C\) is concentration at time \(t\) in nanograms per milliliter, \(A\) is the zero-order intercept in nanograms per milliliter, \(k\) is the slope of the terminal phase of elimination in minutes⁻¹, and \(t\) is time in minutes.

For all methylecgonidine analyses, the terminal linear phase was selected as occurring between 25 to 55 min after methylecgonidine administration. Area under the curve was calculated using the trapezoidal rule. Half-life, clearance, and volume of distribution were calculated using standard pharmacokinetic formulas (Rowland and Tozer, 1995).

For comparison of methylecgonidine half-life to literature values of cocaine half-life, 95% confidence intervals of half-life were computed by transforming the confidence intervals of the slope estimate. RS/1 (Brooks Automation Inc., Chelmsford, MA) calculates the confidence intervals of the slope estimate using the likelihood method,

![Fig. 1. A, cocaine conversion to methylecgonidine and ecgonidine formation from methylecgonidine. B, structures of laboratory synthesized ethylecgonidine and N-ethyl-N-norecgonidine, internal standards for methylecgonidine and ecgonidine plasma assays, respectively.](image-url)
providing a better confidence estimate of a nonlinear function than linearized confidence intervals (Clarke, 1987; Donaldson and Schnabel, 1987).

**Pharmacodynamic Study.** Solutions of methylecgonidine fumarate and AMB were prepared daily in saline. Concentrated methacholine stocks were prepared in saline and stored at \(-80^\circ C\).

Sheep were prepared with indwelling femoral arterial and venous catheters using procedures described above. Sheep were brought to the experimental room and the femoral artery catheter connected to a blood pressure transducer (model 42582-05; Abbott Laboratories, North Chicago, IL). The strain gauge coupler was connected to a tachometer and blood pressure processor (models S77-25, S77-26, and S77-34, respectively; Coulbourn Instruments, Allentown, PA). Heart rate and blood pressure were logged 10 times per second using Biobench version 1.0 (National Instruments, Austin, TX).

Sheep were administered AMB or saline intravenously in a within-animal experimental design (Fig. 2). Two saline vehicle control volumes were used; one was equivalent to the volume of methacholine delivery (approximately 1 ml) and a second equivalent to methylecgonidine fumarate delivery volume (3 ml for 0.1, 0.3, and 1.0 mg/kg; 5 ml for 3.0 mg/kg). Methacholine (5 \(\mu\)g/kg i.v.) served as a positive control for muscarinic agonism. Ten minutes later a dose of methylecgonidine fumarate (0.1, 0.3, 1.0, or 3.0 mg/kg) was administered. Because we hypothesized that methylecgonidine acts as a peripheral muscarinic agonist, we administered AMB, a nonspecific quaternary antagonist with limited central nervous system access. Methacholine and the methylecgonidine study dose were administered after AMB treatment to examine potential antagonism of methylecgonidine effects on heart rate and blood pressure. Experiments using different methylecgonidine doses were conducted at least 48 h apart.

**Pharmacodynamic Data Analysis.** To minimize the impact of movement artifacts, medians were computed from one-second (sheep 4 and 5) or two-second (sheep 3) intervals; data were collected at a resolution of 10 samples/s. Mean arterial pressure (MAP) was computed as diastolic pressure + one-third of diastolic pressure by a blood pressure processor (Coulbourn Instruments). Smoothed curves were fit to heart rate and MAP (Fig. 4) (Chambers et al., 1983).

Effects on heart rate and MAP were expressed as changes from baseline within each experimental session. Baseline MAP was computed as the median of 3 min of MAP data ending 20 s before each drug administration. Baseline heart rate was computed as the median of 2 min of heart rate data ending 20 s before each drug administration.

Cardiovascular effects of methacholine and methylecgonidine were evaluated on four endpoints, which were determined empirically. Bradycardia was defined as the peak decrease in heart rate.
elimination in minutes

analysis of variance; if the analysis of variance was significant at whether methacholine produced an effect on each endpoint in each period from 8 to 28 s after drug administration.

administration. The peak decrease in MAP was evaluated within the computing the median heart rate of a 3- to 5-min window after drug administration. Delayed bradycardia was characterized by evaluated as the peak increase in heart rate observed 10 to 90 s after drug administration. Tachycardia was observed 10 to 25 s after drug administration. Tachycardia was always detectable in the first sample collected, i.e., 30 s to 2 min after injection. Egonidine concentrations increased as methylecgonidine concentrations decreased (Fig. 3). $C_{\text{max}}$ was achieved from 30 to 45 min after injection and seemed to be dose-related (ecgonidine pharmacokinetic data; Table 1). In one 10 mg/kg methylecgonidine session, no decline in ecgonidine concentrations was observed before the last time point (50 min). Note that ecgonidine carryover from previous experiments occurred in four experiments (Table 2). In these cases, the baseline plasma concentrations collected after methylecgonidine administration were dose-related effects, i.e., an effect consistent with muscarinic agonism, the effect of AMB treatment was evaluated by calculating a z-score comparing methylecgonidine effects before ($n = 3$) and after AMB treatment. Similarly, z-score comparisons of methylecgonidine effects before AMB treatment ($n = 3$) and after saline treatment (AMB sham, the third saline in Fig. 2) were computed to determine whether tolerance developed to methylecgonidine.

TABLE 1
Noncompartmental pharmacokinetic parameters of methylecgonidine (MEG) and ecgonidine after intravenous methylecgonidine administration

<table>
<thead>
<tr>
<th>MEG Dose</th>
<th>3.0 mg/kg</th>
<th>5.6 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>MEG</td>
<td>MEG</td>
<td>MEG</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>917.8</td>
<td>1663.9</td>
<td>1239.6</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>2.0</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>$k$</td>
<td>-0.034</td>
<td>-0.033</td>
<td>-0.035</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>20.3</td>
<td>20.7</td>
<td>19.8</td>
</tr>
<tr>
<td>$t_{1/2} CI$</td>
<td>16.3–26.8</td>
<td>17.8–24.7</td>
<td>14.0–33.5</td>
</tr>
<tr>
<td>AUC$_{\text{total}}$</td>
<td>8771</td>
<td>13,683</td>
<td>11,000</td>
</tr>
<tr>
<td>EC</td>
<td>342.0</td>
<td>219.2</td>
<td>509.1</td>
</tr>
<tr>
<td>$V_d$</td>
<td>10.0</td>
<td>6.6</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Methylecgonidine parameters

<table>
<thead>
<tr>
<th>MEG Dose</th>
<th>3.0 mg/kg</th>
<th>5.6 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>EC</td>
<td>Carryover</td>
<td>Experiment</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>1753.0</td>
<td>2218.0</td>
<td>3386.6</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>35</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>$k$</td>
<td>-0.006</td>
<td>-0.007</td>
<td>-0.005</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>113.5</td>
<td>94.4</td>
<td>135.6</td>
</tr>
<tr>
<td>$t_{1/2} CI$</td>
<td>83.4–175.6</td>
<td>59.13–233.8</td>
<td>90.4–271.2</td>
</tr>
<tr>
<td>AUC$_{\text{total}}$</td>
<td>336,465</td>
<td>332,591</td>
<td>635,015</td>
</tr>
</tbody>
</table>

Egonidine parameters

<table>
<thead>
<tr>
<th>MEG Dose</th>
<th>3.0 mg/kg</th>
<th>5.6 mg/kg</th>
<th>10.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>EC</td>
<td>Carryover</td>
<td>Experiment</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>1791.7</td>
<td>2351.9</td>
<td>3905.8</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>45</td>
<td>85</td>
<td>40</td>
</tr>
<tr>
<td>$k$</td>
<td>-0.006</td>
<td>-0.007</td>
<td>-0.005</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>113.5</td>
<td>94.4</td>
<td>135.6</td>
</tr>
<tr>
<td>$t_{1/2} CI$</td>
<td>83.4–175.6</td>
<td>59.13–233.8</td>
<td>90.4–271.2</td>
</tr>
<tr>
<td>AUC$_{\text{total}}$</td>
<td>336,465</td>
<td>332,591</td>
<td>635,015</td>
</tr>
</tbody>
</table>

Note: methylecgonidine was not detected in any baseline samples.

TABLE 2
Egonidine (EC) carryover from previous methylecgonidine (MEG) experiments

Note: methylecgonidine was not detected in any baseline samples.

<table>
<thead>
<tr>
<th>Previous MEG Experiment</th>
<th>Sheep</th>
<th>EC$_{50}$ min $^a$</th>
<th>EC Carryover</th>
<th>MEG Experiment $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 mg/kg, 2 days prior</td>
<td>1</td>
<td>3071.4</td>
<td>262.42</td>
<td>10.0</td>
</tr>
<tr>
<td>3.0 mg/kg, 2 days prior</td>
<td>2</td>
<td>2579.2</td>
<td>327.70</td>
<td>3.0</td>
</tr>
<tr>
<td>10.0 mg/kg, 4 days prior</td>
<td>1</td>
<td>5949.3</td>
<td>50*</td>
<td>5.6</td>
</tr>
<tr>
<td>10.0 mg/kg, 4 days prior</td>
<td>2</td>
<td>5106.0</td>
<td>167.22</td>
<td>3.0</td>
</tr>
</tbody>
</table>

$^a$ Egonidine concentration of the sample collected 50 min after methylecgonidine bolus, 2 or 4 days before collection of baseline sample containing ecgonidine.

$^b$ Methylecgonidine experiment refers to the experimental session for which the baseline sample contained ecgonidine.

ND, ecgonidine concentration was less than the level of detection (10 ng/ml).

TABLE 3
Egonidine (EC) plasma concentrations 24 and 48 h after methylecgonidine (MEG) injection

Note: methylecgonidine was not detected in any samples at 24 or 48 h.

<table>
<thead>
<tr>
<th>Animal</th>
<th>MEG Dose</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>ng/ml</td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
<td>1663.5</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>2882.8</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>4638.9</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, maximum concentration in nanograms per milliliter; $T_{\text{max}}$, time in minutes that maximum concentration occurred; $k$, slope of the line fit to the terminal phase of elimination in minutes $^a$; $t_{1/2}$, half-life of elimination in minutes; $t_{1/2} CI$, 95% confidence intervals of half-life; AUC$_{\text{total}}$, area under the curve in nanograms $\times$ minutes $\times$ milliliters $^b$; CL, clearance in milliliters $\times$ kilograms $^{-1} \times$ minutes $^{-1}$; $V_d$, volume of distribution of the terminal phase in liters $\times$ kilograms $^{-1}$.

$^a$ No decline in ecgonidine levels.

Results

Methylecgonidine quickly achieved maximal plasma concentration ($C_{\text{max}}$) and was subsequently rapidly cleared from blood for all three doses (Fig. 3 and Table 1). Samples collected at 24 and 48 h after methylecgonidine injection never contained detectable concentrations of methylecgonidine.

Egonidine occurred in plasma immediately and was always detectable in the first sample collected, i.e., 30 s to 2 min after injection. Egonidine concentrations increased as methylecgonidine concentrations decreased (Fig. 3). $C_{\text{max}}$ was achieved from 30 to 45 min after injection and seemed to be dose-related (ecgonidine pharmacokinetic data; Table 1). In one 10 mg/kg methylecgonidine session, no decline in ecgonidine concentrations was observed before the last time point (50 min). Note that ecgonidine carryover from previous experiments occurred in four experiments (Table 2). In these cases, the baseline plasma concentrations were subtracted from measured ecgonidine concentrations collected after methylecgonidine adminis-
tration, and the corrected values were used for all subsequent data analysis. Ecgonidine was present in all 24-h samples that were collected (Table 3). Ecgonidine elimination was prolonged relative to methylecgonidine with 3-fold increase in half-lives of ecgonidine relative to methylecgonidine.

**Effects of Methacholine.** A representative recording from an experimental session illustrating effects of methacholine on heart rate and mean arterial pressure before and after AMB treatment is presented in Fig. 4, A and C. Methacholine induced significant, rapid-onset bradycardia occurring 8 to 20 s after administration (Fig. 5A). Intravenous 15 \( \mu \)g/kg AMB treatment 10 min before the second methacholine dose significantly attenuated bradycardia in each animal.

Figure 5B illustrates the effects of AMB on methacholine-induced changes in mean arterial blood pressure. In all three sheep, 5 \( \mu \)g/kg methacholine i.v. caused a significant drop in mean arterial pressure, occurring within 8 to 20 s of drug delivery. Comparison of the average from four sessions for maximal decrease in MAP produced by methacholine, before and after AMB treatment, revealed significant antagonism for two of three sheep.

Significant tachycardia occurred within 20 to 60 s of methacholine administration. Tachycardia occurred after the initial bradycardia and nearly superimposed with hypotension (Fig. 5C). This tachycardia was antagonized with AMB in two of the three animals. Methacholine did not produce any significant delayed bradycardia (3–5 min after methacholine administration) in any of the sheep (Fig. 5D).

**Effects of Methylecgonidine.** A representative recording of the effects of 1.0 mg/kg methylecgonidine on heart rate and mean arterial pressure before and after 15 \( \mu \)g/kg AMB treatment is presented in Fig. 4, B and D. Methylecgonidine administration produced hypotension and tachycardia. There was no bradycardia immediately after methylecgonidine administration, as there was for methacholine. Analysis of the minimum heart rate 10 to 25 s after methylecgonidine administration demonstrated tachycardia (Fig. 6A).

Significant tachycardia occurred in all three sheep 10 to 90 s after methylecgonidine administration. Methylecgonidine tachycardia dose-effect curves exhibited inverted U-shaped responses in two of three sheep (Fig. 6B).

In two of three sheep, bradycardia occurred 3 to 5 min after methylecgonidine administration (Fig. 6C). However, two of three saline sham control experiment results demonstrated significant differences from methylecgonidine baseline values, so effects of AMB treatment on this endpoint were not evaluated.

In all three sheep, methylecgonidine-induced hypotension (Fig. 7). Pretreatment with 15 \( \mu \)g/kg AMB 20 min before methylecgonidine administration shifted the dose-response curve to the right, consistent with antagonism. To establish that the AMB antagonism was surmountable (i.e., competitive) in sheep 3 and 4, the AMB dose was decreased to 7 and 4 \( \mu \)g/kg, respectively. Saline (sham antagonist) experiments provided no evidence of tolerance to methylecgonidine-induced hypotension.

**Discussion**

This work comprises the first description of pharmacokinetic data of methylecgonidine clearance from blood, demonstrating that methylecgonidine is quickly cleared from blood.
but has a longer half-life than cocaine in sheep based upon published cocaine values (Khan et al., 1987). The apparent volume of distribution of methylecgonidine is larger than that for cocaine in sheep. Therefore, the low methylecgonidine concentrations in forensic blood specimens seems largely due to methylecgonidine's rapid distribution and metabolism. Ecgonidine is a metabolite of methylecgonidine, in vivo, persists longer than methylecgonidine, and should prove to be a more useful indicator of crack smoking than methylecgonidine. Methylecgonidine induces rapid-onset hypotension that is competitively antagonized by atropine methyl bromide, supporting the hypothesis that methylecgonidine can act as a muscarinic agonist, in vivo.

Methylecgonidine is detected infrequently in blood or plasma samples and has never been detected in blood samples collected from crack smokers in controlled settings (Cone et al., 1994; Jenkins et al., 1995). A hypothesis that methylecgonidine is cleared quickly from blood is a possible explanation. Rapid clearance could suggest that proper sample handling and storage conditions are vital in the pharmacokinetic studies reported here. Previous work demonstrated that methylecgonidine is more stable than cocaine in sheep and human plasma; freezing samples at −80°C provides adequate methylecgonidine storage for several months (Scheidweiler et al., 2000; Fandino et al., 2002). Thus, in vitro hydrolysis of methylecgonidine to ecgonidine is not likely to occur and would not confound methylecgonidine or ecgonidine pharmacokinetic analysis. Undetectable methylecgonidine concentrations observed in clinical studies may result from methylecgonidine's rapid clearance from the central compartment, additionally complicated by low levels of exposure to methylecgonidine from smoking in experimental contexts.

It is difficult to determine a standard methylecgonidine exposure from crack smoking, because there is much variability observed in methylecgonidine formation from cocaine and formation is temperature-dependent. Nakahara and Ishigami (1991) reported that 68% of cocaine base is converted to methylecgonidine at 320°C and under vacuum, conditions not achieved in a crack pipe. Heating of cocaine base in a model crack pipe with a Bunsen burner at atmospheric pressure typically demonstrated less than 2% methylecgonidine conversion from cocaine, with a maximum of 5% observed when the cocaine ignited (Wood et al., 1996). Because we wished to determine pharmacokinetic and cardiovascular responses to methylecgonidine, the concentrations of methylecgonidine here are most likely higher than those attained from typical crack smoking.

Both methylecgonidine and cocaine have apparent volumes of distribution larger than the blood volume of sheep, 64.9 ml/kg. This indicates that methylecgonidine, like cocaine, distributes from blood to other tissues. The mean volume of distribution of 4 mg/kg cocaine in sheep is 3.11 l/kg, and the volumes of distribution of 3 mg/kg methylecgonidine for sheep 1 and 2 were 10.0 and 6.6 l/kg, respectively (Khan et al., 1987). Calculation of z-scores confirms that both sheep

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Fig. 5. Effects of 5 µg/kg methacholine on heart rate and MAP (n = 4, mean ± S.E.M.). Effects of 15 µg/kg atropine methyl bromide pretreatment 20 min before methacholine administration are also shown. A, methacholine effect on peak decrease in heart rate 8 to 28 s after administration. B, effect on peak decrease in mean arterial pressure 10 to 25 s after administration. C, effect on peak heart rate occurring 30 to 90 s after administration. D, effect on median heart rate occurring 3 to 5 min after administration. *, p < 0.05 and **, p < 0.005, significant differences by paired t test.
Methylecgonidine produces effects consistent with muscarinic agonism in the awake sheep. Like methacholine, intravenous methylecgonidine administration produces dose-related decreases in mean arterial pressure in conscious sheep that can be antagonized with AMB. Antagonism of methylecgonidine-induced hypotension by AMB was surmountable, consistent with atropine’s competitive antagonism of other muscarinic effects (Brown and Taylor, 1996).

There are two possible mechanisms for muscarinic induced hypotension. Hypotension has been linked to stimulation of M₄ receptors in the vascular endothelium, which stimulates nitric-oxide synthase to produce nitric oxide, thereby relaxing the underlying smooth muscle (Lind et al., 1999; Sawyer et al., 1999). Additionally M₄ receptor stimulation decreases cardiac contractile force via inhibition of adenylyl cyclase and stimulation of guanylyl cyclase, which could also produce a drop in blood pressure (Kelly et al., 1996).

This study was unable to demonstrate significant bradycardia immediately after methylecgonidine administration. This contrasted with methacholine, which produced marked bradycardia. The lack of methylecgonidine-induced bradycardia seems to contrast with reports of in vitro M₂ agonist properties in myocardium (Woolf et al., 1997), myocytes (Huang et al., 1997; Yang et al., 2002), and human embryonic lung cells expressing M₂ receptors (Yang et al., 2001). Data from these reports relate to muscarinic signaling pathways involved in decreasing contractility and vasodilation. Muscarinic-induced bradycardia is caused by M₂ stimulation of the acetylcholine-activated potassium current, K_{ACCh}, which delays cardiac depolarization (Yamada, 2002). In vitro studies did not show methylecgonidine to possess any agonist effects on K_{ACCh} in myocytes (Xiao and Morgan, 1998).

Mild bradycardia was present 3 to 5 min after 1.0 and 3.0 mg/kg methylecgonidine administration. However, there was not any observed bradycardia after methacholine at the same time interval. The kinetics of association of methylecgonidine to M₂ muscarinic receptors is unknown. Methylecgonidine might have slower receptor kinetics than methacholine.

Tachycardia was observed after both methacholine and methylecgonidine administration. Methacholine-induced tachycardia may result from muscarinic-mediated bronchoconstriction, because the dose of methacholine consistently induced coughing in two of three sheep. Mild coughing was only observed with the highest dose of methylecgonidine. Tachycardia may also result via baroreceptor compensation to decrease in MAP, because tachycardia did occur with a similar time course as decrease in MAP for methylecgonidine.

A number of cardiovascular disorders have been documented in cocaine users, including hypertension, arrhythmias, and infarction (Knuepfer, 2003). This work does support the hypothesis that methylecgonidine may play a role in cardiovascular toxicity associated with crack use (Yang et al., 2002). Our results confirm that methylecgonidine does produce hypotension, an effect consistent with methylecgonidine stimulation of nitric-oxide synthase as shown in cell preparations (Yang et al., 2001, 2002). It is possible that methylecgonidine stimulation of nitric-oxide synthase leads to the production of reactive oxygen species that play a role in cardiovascular toxicity resulting from crack use.

Muscarinic receptors play an important role in cognition as evidenced by cognitive impairments observed after treatment with scopolamine, a nonspecific muscarinic antagonist. Scopolamine treatment impairs performance of rats in water and radial-arm mazes (Cassel and Kelche, 1989; Saucier et al., 1996). Impaired working memory was reported in monkeys after scopolamine treatment (Schwarz et al., 1999). Additionally, long-term exposure to muscarinic agonists has
been demonstrated to cause down-regulation of muscarinic receptors (Haddad el-B and Rousell, 1998).

Some studies have demonstrated cognitive impairments in crack users. Impaired executive function and memory were seen in a group of 30 cocaine users, 29 of whom reported crack as their preferred form of cocaine use (Bolla et al., 1999). DiSclafani et al. (2002) also reported impaired executive function after 6 weeks of abstinence in crack, and in crack- and alcohol-dependent subjects; the strongest predictor of neuropsychological impairment in the latter study was dose. If methylecgonidine is acting as a muscarinic agonist in vivo, it is possible that repeated methylecgonidine exposure is playing a role in crack-associated cognitive deficits by causing down-regulation of muscarinic receptors in brains of crack smokers. However, this work also demonstrates that any consideration of methylecgonidine as a toxicant should be tempered by the understanding that methylecgonidine is causing down-regulation of muscarinic receptors in brains of crack smokers. However, this work also demonstrates that activity of methylecgonidine, the more persistent indicator of methylecgonidine exposure/crack smoking. Additionally, our data are the first to demonstrate muscarinic agonism by methylecgonidine in vivo.

References


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