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Drug Monitoring and Toxicology

Methamphetamine and Amphetamine Pharmacokinetics in Oral Fluid and Plasma after Controlled Oral Methamphetamine Administration to Human Volunteers

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Background: **Methamphetamine (METH) and amphetamine (AMP) concentrations in 200 plasma and 590 oral fluid specimens were used to evaluate METH pharmacokinetics and pharmacodynamics after oral administration of sustained-release METH.**

Methods: **Eight participants received four oral 10-mg *S*-(+)-METH hydrochloride sustained-release tablets within 7 days. Three weeks later, five participants received four oral 20-mg doses. Blood samples were collected for up to 24 h and oral fluid for up to 72 h after drug administration.**

Results: **After the first oral dose, initial plasma METH detection was within 0.25–2 h; c_{max} was 14.5–33.8 μg/L (10 mg) and 26.2–44.3 μg/L (20 mg) within 2–12 h. In oral fluid, METH was detected as early as 0.08–2 h; c_{max} was 24.7–312.2 μg/L (10 mg) and 75.3–321.7 μg/L (20 mg) and occurred at 2–12 h. The median oral fluid-plasma METH concentration ratio was 2.0 across 24 h and was highly variable. Neutral cotton swab collection yielded significantly higher METH and AMP concentrations than citric acid candy-stimulated expectoration. Mean (SD) areas under the curve for AMP were 21% ± 25% and 24% ± 11% of those observed for METH in plasma and oral fluid, respectively. After a single low or high dose, plasma METH was >2.5 μg/L for up to 24 h in 9 of 12 individuals (mean, 7.3 ± 5.5 μg/L at 24 h); in oral fluid the detection window was at least 24 h (mean, 18.8 ± 18.0 μg/L at 24 h). The plasma and oral fluid 24-h METH detection rates were 54% and 60%, respectively. After four administrations, METH was measurable for 36–72 h (mean, 58.3 ± 14.5 h).**

Conclusions: **Perceived advantages of oral fluid for verifying METH exposure compared with urine include simpler specimen collection and reduced potential for adulteration, but urine offers higher analyte concentrations and a greater window of detection.**

Methamphetamine (METH),[4] a potent, highly addictive stimulant, was synthesized for therapeutic use in the early 1900s *(1)*. METH abuse is a serious problem in the US, Mexico, South America, the Middle East, the Arabian peninsula, Asia, and Australia *(2–4)*.

METH, like other amphetamines, is a sympathomimetic drug. When administered intravenously, small doses have prominent central stimulant effects *(5–7)*. In humans, METH is readily absorbed from the gastrointestinal tract after oral consumption *(8)*. METH is almost entirely (90%) eliminated in urine *(9)*. In urine of pH 6–8, ~22% of a METH dose is excreted as unchanged drug, 15% as *p*-hydroxymethamphetamine (pOH-METH), 4–7% as amphetamine (AMP), and 1% as *p*-hydroxyamphet-

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[4] Nonstandard abbreviations: METH, methamphetamine; pOH-METH, *p*-hydroxymethamphetamine; AMP, amphetamine; pOH-AMP, *p*-hydroxyamphetamine; TDM, therapeutic drug monitoring; S-P ratio, oral fluid-plasma ratio; NIDA, National Institute on Drug Abuse; MTBSTFA, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide; TBDMS, *tert*-butyldimethylsilyl; TMS, trimethylsilyl; LOD, limit of detection; LOQ, limit of quantification; $AUC_{0-24\ h}$, area under the curve from the time of dosing to the time of the last observation (24 h); and SAMHSA, Substance Abuse and Mental Health Services Administration.

amine (pOH-AMP) *(8, 9)*. Of quantitatively minor importance are phenylacetone and the hydroxylation products of AMP and pOH-AMP, i.e., norephedrine and *p*-hydroxynorephedrine *(10)*. During phase two biotransformation, the majority of pOH-METH and pOH-AMP is conjugated with glucuronic acid *(9)*. The renal excretion of METH and AMP is enhanced by urinary acidification, producing shortened plasma elimination half-lives and increased total clearance *(11)*.

Oral fluid is an alternative biological matrix to urine and plasma for drugs-of-abuse testing *(12)*. Interest has increased because of the ease, noninvasiveness, and safety of specimen collection. Today, its usefulness as an aid in clinical diagnosis and for therapeutic drug monitoring is established *(12)*. Indeed, oral fluid testing has been successfully used as an alternative to blood testing in pharmacokinetic and pharmacotoxicologic studies; in many cases, drug in oral fluid represents the physiologically active fraction *(13, 14)*.

For therapeutic drug monitoring (TDM) purposes, the usefulness of oral fluid as a test matrix is dependent on consistent oral fluid-plasma (S-P) ratios *(15)*. For oral fluid concentrations to accurately predict plasma concentrations, the S-P ratio must be independent of drug concentration and consistent within and across individuals. In practice, oral fluid is used only to monitor a select array of drugs because of large intra- and intersubject variability in S-P ratios. This variability can be explained by a multiplicity of factors that control drug disposition from plasma. In theory, a drug circulating in plasma must cross the capillary membrane, the basal membrane, and glandular epithelial cells of the salivary gland before it can pass into oral fluid. The mechanisms involved in these distribution processes remain largely unknown for many substances, including METH and AMP. One or more of the following transport processes could be involved in the transport of METH and AMP from plasma to oral fluid: (*a*) passive diffusion, (*b*) facilitated diffusion (a carrier-mediated, non-energy-consuming process), or (*c*) a carrier-mediated active transport mechanism. As opposed to TDM, the use of oral fluid for drug detection is not dependent on predicting plasma concentrations; rather it is used to detect drug use. Therefore, S-P ratios are less important.

The pharmacokinetics of METH and AMP in plasma and oral fluid in humans after controlled METH administration have been described in only two reports, both from Cook and coworkers *(16, 17)*: one after oral METH administration *(16)*, and the other after METH vapor inhalation *(17)*. Other reports providing information about METH pharmacokinetics do not include oral fluid data or the effect of different oral fluid collection methods on METH drug concentrations. Shappell et al. *(18)* correlated plasma METH concentrations with chronopharmacodynamic data. Perez-Reyes and coworkers *(19, 20)* focused on the pharmacodynamic data from the above-mentioned clinical studies conducted by Cook and coworkers *(16, 17)*. Driscoll et al. *(21)* determined the mean blood METH concentrations of 10 females after oral METH intake, and Suzuki et al. *(22)* analyzed METH and AMP in various biological matrices, including 20 oral fluid specimens collected from drug abusers.

The aims of this study were to describe the pharmacokinetics of METH and AMP in human oral fluid and plasma after controlled oral METH administration, to determine whether oral fluid concentrations can be used to predict plasma concentrations, and to compare oral fluid collection methods. In addition, these METH and AMP concentration data will strengthen the interpretation of oral fluid test results and will aid in establishing the detection window of METH in oral fluid. Concurrent pharmacodynamic effects of oral METH administration were also investigated.

Materials and Methods

PARTICIPANTS AND STUDY DESIGN

The study was approved by the National Institute on Drug Abuse (NIDA) Institutional Review Board. Throughout the study, participants resided in the Intramural Research Program's secure clinical ward at NIDA. All participants provided written informed consent and were paid for their participation.

Before admission, each participant underwent thorough medical, physical, and psychologic evaluations, including a history of past and recent drug use. Participants resided on a closed clinical ward for 2 weeks before METH administration to permit elimination of previously self-administered drugs.

Participants received four daily 10-mg (low) oral *S*-(+)-methamphetamine · HCl doses within 7 days. After a 3-week interval, five of eight participants received four daily 20-mg (high) oral doses of *S*-(+)-methamphetamine · HCl. Two participants were disqualified from the study for medical reasons. A third participant chose to discontinue the study after the fourth dose. Participants received a single gelatin capsule containing one or two Desoxyn® Gradumet® 10-mg sustained release tablets (Abbott Laboratories) with lactose (Amend Drug & Chemical Co., Inc.) as the filler. For placebo treatments, the capsule contained lactose only.

All drug administrations were conducted under subject-blind conditions. The Gradumet formulation was developed to sustain a slow release of drug after oral administration. Dissolution tests of the preparation showed that 20–40% of the drug is released after 30 min, 35–55% after 1 h, and 60–80% after 4 h. Desoxyn Gradumet 5 mg tablets are currently available.

METH is commonly abused via smoking, insufflation, injection, and less commonly, via the oral route *(23)*. In our controlled administration study in human volunteers, a sustained-release oral METH formulation was used because of health and safety concerns for study participants.

SPECIMEN COLLECTION

Participants consumed a light breakfast in the morning and did not smoke or eat for 1 h before the session. Oral fluid was collected 15 min before; 5, 10, 15, and 30 min; and 1, 2, 4, 8, 11.5, and 24 h after drug administration. Three oral fluid collection methods were used. During the first two sessions, a citric acid sourball candy with 90.3 ± 3.3 mg citric acid (Brach's Confections Inc.) stimulated oral fluid production. Participants expectorated ~5 mL of oral fluid into a 50-mL screw cap polypropylene collection tube. During session three, oral fluid was collected by placing a cotton swab treated with 20 mg of citric acid (Salivette® cotton swab citric acid preparation; Sarstedt International) in the volunteer's mouth. During the fourth session, cotton swabs without citric acid (Salivette cotton swab without preparation; Sarstedt) were used for oral fluid collection. The liquid was extracted from the swab by centrifugation. Samples were transferred into polypropylene tubes and stored at −20 °C until further analysis.

Venous blood was drawn from the forearm through a heparin lock and transferred to a sodium heparin-containing Vacutainer® (Becton Dickinson) to which 87 μL of a saturated sodium fluoride solution and 87 μL of a solution of 100 mL/L glacial acetic acid in water were added. After centrifugation, plasma was isolated, and specimens were stored in a polypropylene tube at −20 °C until analysis. During the first session of both the low- and high-dosage regimens, blood samples were collected simultaneously with oral fluid for up to 24 h. During all other sessions, a single blood sample was collected 24 h after drug administration. For the well being of participants, limitations were imposed on the total amount of blood that could be collected during the study.

COLLECTION OF PHARMACODYNAMIC DATA

Physiologic data were collected during the first low session, first high session, and a placebo session with a Datascope® Passport® Model EL physiologic monitor (Datascope Corporation). Pupillometry was assessed with use of an adapted Polaroid camera. Within each session, systolic and diastolic blood pressure, heart rate, respiration rate, oxygen saturation, skin and core temperature, and pupil diameter were recorded at baseline (15 min before dosing); 5, 10, 15, 30, 45, 60, 75, 90, and 105 min; and 2, 4, 8, and 11.5 h after drug administration. No physiologic data were collected for volunteer V.

SPECIMEN ANALYSIS

Samples were analyzed for METH and AMP by solid-phase extraction and gas chromatography–mass spectrometry procedures similar to those described by Huestis et al. *(24)* for the simultaneous determination of opiates, cocaine, and their metabolites. The method of analysis was developed further to simultaneously determine METH, cocaine, codeine, and their metabolites so that a single analytical method could be used for the analysis of samples collected in multiple drug administration studies.

To 1 mL of specimen were added 100 μL of a 1 μg/L aqueous solution of internal standards (d_{11}-METH and d_{10}-AMP) and 3 mL of 2.0 mol/L sodium acetate buffer (pH 4.0) in a 4-mL fritted reservoir (UCT Inc.). Samples were allowed to stand for 10 min and were centrifuged at 3000*g* for 5 min. The supernatant was collected in 16 × 100 mm glass test tubes and decanted onto CSDAU Clean Screen Extraction columns (UCT Inc.) preconditioned with 1 mL of a mixture of methylene chloride, 2-propanol, and 14.5 mol/L ammonium hydroxide (80:20:2 by volume) followed by 3 mL of methanol, 3 mL of deionized water (twice), and 1.5 mL of 2 mol/L sodium acetate buffer (pH 4). Vacuum was applied, and columns were washed sequentially with two 1-mL aliquots of water, 1.5 mL of 0.1 mol/L HCl, and two 1-mL aliquots of methanol.

After the solid-phase extraction columns were dried, analytes were eluted four times with 1 mL of a mixture of methylene chloride, isopropanol, and 14.5 mol/L ammonium hydroxide (80:20:2 by volume). The combined eluates were collected in conical glass centrifuge tubes with 20 μL of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide containing 10 g/L *tert*-butyldimethylchlorosilane (MTBSTFA +1% TBDMCS; Pierce) to reduce METH and AMP volatility. Samples were then evaporated under a continuous nitrogen stream in a water bath at 40 °C until dry. A 500-μL aliquot of acetonitrile was added, and tubes were vortex-mixed to recover drug from centrifuge tube walls. After samples were evaporated to dryness, 20 μL of acetonitrile was added and centrifuge tubes were capped, vortex-mixed, and centrifuged at 3000*g* for 5 min. Samples were transferred to autosampler vials, and 20 μL of MTBSTFA was added. Vials were loosely capped and placed in a heat block at 80 °C for 15–20 min. We then added 20 μL of *N,O*-bis(trimethylsilyl)trifluoroacetimide containing 10 g/L trimethylchlorosilane (BSTFA +1% TMCS; Pierce), crimp-capped the vials, and heated them at 80 °C for 45 min.

Analyses were performed on a Hewlett-Packard Model 6890 gas chromatograph equipped with a Hewlett-Packard Model 6890 autosampler and a 12 m × 0.2 mm (i.d.) HP-1 capillary column (0.33 μm film thickness) or a 15 m × 0.2 mm (i.d.) Phenomenex® ZB1 capillary column (0.10 μm film thickness) interfaced with a Hewlett-Packard Model 5973A electron impact mass spectrometer. Helium was used as carrier gas (flow rate, 1 mL/min) for 1-μL splitless sample injections. The initial oven temperature was 70 °C with a 1-min hold followed by ramps at 35 °C/min to 200 °C, 25 °C/min to 250 °C, and 21 °C/min to 325 °C with a 1-min final hold. Total run time was ~11 min. Specimens were analyzed in selected-ion monitoring mode for the following ions (where q is the quantitative ion): d_{11}-METH [trimethylsilyl (TMS) derivative], *m/z* 136 (q), 96, and 217; METH (TMS derivative), *m/z* 130 (q), 91, and 206; d_{10}-AMP [*tert*-butyldimethylsilyl (TBDMS) derivative], *m/z* 162 (q), 202, and 244; AMP (TBDMS deriv-

ative), *m/z* 158 (q), 192, and 234. Samples were accepted for quantification if both target ion ratios were within ± 20% of the values computed for the 10 μg/L calibrator (100 μg/L internal standard). In addition, chromatography had to be acceptable for all ions, and retention times had to be within ± 2%.

A total of 590 oral fluid and 200 plasma specimens were analyzed. METH and AMP peak areas were integrated using the HP ChemStation Software® (Rev. C.00.00), and the ratio of the area of the calibrator and its internal standard were used for calculations. For each sample batch, two calibration curves, i.e., 2.5–50 and 50–500 μg/L, were generated to obtain adequate sensitivity and linearity ($r > 0.993$). The limits of detection (LOD) and quantification (LOQ) were 2.5 μg/L for both analytes. LODs and LOQs were determined by analyzing serially diluted blank oral fluid specimens to which METH and AMP had been added. The LOD of the analyte was based on its correct retention time, a signal-to-noise ratio for all ions of 3:1, and qualifier ratios within ± 20% of those observed with a 10 μg/L calibrator. In addition to the LOD criteria, the LOQ required quantification within 20% of the target concentration for three of four replicates. Duplicate control samples at 12.5, 25 and 250 μg/L were included in each batch and were required to be within 20% of their theoretical value. Between-run imprecision, as measured with duplicate control samples at 12.5, 25, and 250 μg/L (n = 10 sets of duplicates), was 12%, 10%, and 5.3% for oral fluid METH and 7.5%, 7.8%, and 4.2% for oral fluid AMP, respectively; the between-run imprecision for plasma was 4.3%, 3.5%, and 1.4% for METH and 1.7%, 2.8%, and 1.9% for AMP for the same set of controls, respectively (n = 7 sets of duplicates).

pH measurements of oral fluid specimens

The pH of oral fluid specimens was measured using pH indicator sticks (JT Baker). Small aliquots of oral fluid samples were spotted on the pH indicator sticks, and the color of the sticks was compared with a color scale. The pH resolution was ≤0.4 pH units.

pharmacokinetics and statistical analysis

The pharmacokinetics of METH and AMP were evaluated by use of WinNonlin® (Pharsight Corporation). Noncompartmental analysis included determination of the following parameters: t_{lag}, corresponding to the time before the first measurable (non-zero) concentration; t_{max}, the time to maximum observed concentration; c_{max}, the concentration corresponding to t_{max}; $t_{1/2}$, estimated via linear regression of time vs the log terminal end portion of the curve; and $AUC_{0-24\ h}$, the area under the curve from the time of dosing to the time of the last observation (24 h). Volume of distribution (V_d) values were expressed in L/kg of body weight and were computed based on the terminal elimination phase; Cl represents the total body clearance for extravascular administration. AUCs were calculated by the linear trapezoidal rule. Data are reported as the mean ± SD unless otherwise defined. Plasma data from individual participants were evaluated with a one-compartment model with first-order input and output and computation of absorption (K_{01}) and elimination constants (K_{10}). The evaluation of best fit was based on examination of the CVs for the parameter estimates and on the Akaike criterion *(25)*.

Plasma METH concentrations after the first low and high dose were compared using a paired Student *t*-test of their respective log-normalized AUC values. To investigate the effect of dose and collection device (i.e., the fixed effects) on oral fluid METH concentrations while taking into account random subject effects, we used SAS® software incorporating a repeated-measures mixed-effects ANOVA. Post hoc analysis of these data with the Scheffé pairwise test enabled us to verify sign and significance of the identified effects.

Similarly, to investigate the effects of subject (random variable), dose, and collection device (fixed effects) on oral fluid pH, we used SAS software incorporating the General Linear Model procedure for mixed-model ANOVA. Post hoc analysis of the data with the Scheffé pairwise test gave sign and significance of the identified effects.

For the analysis of physiologic data, the AUC values of the time–effect curves were calculated. The effect of METH dose on the physiologic response was investigated by use of a one-way ANOVA. Post hoc analysis was performed with the Bonferroni test, allowing for pairwise comparisons of low dose, high dose, and placebo physiologic parameter AUCs.

All data were screened for outliers by use of box plots. No outliers were detected except for S-P ratios, which were subsequently subjected to nonparametric testing. Thus, all data were included in all calculations. The α level was set at 0.05 for all analyses. Effects were considered statistically significant at $P < 0.05$.

Results

participant data

Four males and four females participated in the study. Their mean age was 35.3 ± 4.2 years (range, 26.3–39.8 years); their mean height was 171.5 ± 9.8 cm (range, 149.9–177.8 cm), and their mean weight was 72.0 ± 17.6 kg (range, 54.7–103.2 kg). All eight participants received four low doses, and volunteers S, W, Y, AA, and BB (n = 5) received four high doses. No plasma data were available for volunteer Z after the first low dose because of blood collection difficulties.

meth and amp pharmacokinetics in plasma and oral fluid

Results for plasma and oral fluid METH after the first low and high METH doses are summarized in Table 1 and are presented graphically in Fig. 1. METH was initially detected in plasma after both doses within 0.25–2.0 h (n = 12). After the first low dose (n = 7), maximum METH

Table 1. Noncompartmental analysis of 24-h plasma and oral fluid METH concentration data from session one after administration of single 10- and 20-mg doses of oral sustained-release METH.[a]

	METH dose			
	10 mg (n = 8)[b]		20 mg (n = 5)	
METH	Mean ± SD	Range	Mean ± SD	Range
Plasma				
t_{lag}, h	1.5 ± 0.8	0.3–2.0	1.1 ± 0.5	0.5–2.0
t_{max}, h	5.4 ± 2.5	2.0–8.0	7.5 ± 3.4	2.0–11.5
c_{max}, μg/L	20.2 ± 6.4	14.5–33.8	32.4 ± 7.7	26.2–44.3
$t_{1/2}$, h	9.3 ± 3.7	2.1–14.0	11.1 ± 7.2	2.2–21.2
$AUC_{0-24\ h}$, h · μg/L	269.1 ± 94.3	84.0–357.2	468.1 ± 151.8	258–621.3
V_d,[c] L/kg	5.8 ± 2.6	1.6–8.9	5.3 ± 2.1	1.7–6.7
Cl,[d] L/h	32.2 ± 13.7	22.9–62.4	33.5 ± 15.9	21.0–55.4
Oral fluid				
t_{lag}, h	0.6 ± 0.6	0.1–2.0	0.4 ± 0.1	0.3–0.5
t_{max}, h	5.0 ± 1.9	4.0–8.0	4.7 ± 3.9	2.0–11.5
c_{max}, μg/L	106.1 ± 100.8	24.7–312.2	192.2 ± 120.8	75.3–321.7
$t_{1/2}$, h	7.1 ± 2.3	3.2–10.9	8.1 ± 1.9	
$AUC_{0-24\ h}$, h · μg/L	1118.0 ± 983.4	434.8–2803.7	1874.9 ± 1237.6	5.5–10.5
V_d, L/kg	1.7 ± 0.9	0.6–3.2	2.6 ± 2.1	673.8–3891.0
Cl, L/h	13.2 ± 6.5	3.4–19.6	12.9 ± 7.5	4.5–24.7

[a] Citric acid candy was used to stimulate expectoration.
[b] No plasma samples were collected from volunteer Z.
[c] Volume of distribution.
[d] Total body clearance for extravascular administration.

concentrations averaged 20.2 ± 6.4 μg/L (range, 14–34 μg/L) within 2–8 h. After the first high dose (n = 5), peak plasma METH concentrations averaged 32.4 ± 7.7 μg/L (range, 26–44 μg/L) and occurred at 2–12 h post dose. The 24-h AUCs for plasma METH after the first low and high dose were significantly dose dependent and were, on average, 2.1 ± 0.7 (range, 1.2–3.1) times higher after the high dose than after the low dose ($P = 0.009$).

After a single 10-mg dose, AMP was detected in plasma in five of eight participants (Table 2). AMP was initially detected 1–12 h after drug administration. AMP concentrations reached their maximum at 8–24 h and averaged 4.7 ± 2.5 μg/L (range, 3–8 μg/L). After the first high dose, AMP was initially detected in all participants' specimens at 2–12 h and peaked 12–24 h post dose, averaging 5.6 ± 3.2 μg/L (range, 3–11 μg/L). The mean 24-h AUCs for plasma AMP after the first low and high doses were 40 ± 33 and 81 ± 44 h · μg/L (n = 5), respectively, and on average 24-h AUCs for plasma AMP were 21% ± 25% of those observed for the parent drug METH on a molar basis.

When we fit plasma METH concentration data with a one-compartment model with lag time and first-order kinetics, the absorption (K_{01}) and elimination (K_{10}) constants were estimated at K_{01} = 1.07 ± 0.60 h and K_{10} = 10.76 ± 4.20 h (n = 13) with an acceptable uncertainty (CV <42%).

METH was initially detected in oral fluid 0.08–2 h after the first low (n = 8) or high (n = 5) dose. After the first low dose, mean peak METH concentrations were 106.1 ± 24.7 (range, 25–312) μg/L, occurring at 4–8 h post dose. After the first high dose, peak concentrations were observed 2–12 h post dose and averaged 192.2 ± 120.8 (range, 75–322) μg/L.

After administration of the first low dose, AMP was detected in oral fluid of only five of eight participants in specimens collected at 0.5–4 h (Table 2). Maximum AMP concentrations averaged 8.6 ± 6.5 (range, 4–21) μg/L and were detected in specimens collected 4–12 h after administration. After a single high dose, AMP was initially detected in all participants' oral fluid 1–12 h post dose, with peak concentrations averaging 14.3 ± 6.1 (range, 3–20) μg/L 2–12 h after drug administration. Oral fluid AMP AUCs were on average 24.0% ± 11.4% of those observed for METH on a molar basis, across low and high dose administrations.

Oral fluid METH concentrations were higher than those in plasma, with the mean oral fluid AUCs being 3.8 ± 2.3 (range, 1.8–8.7) times higher ($P = 0.009$) than the plasma AUCs.

The S-P ratios for METH were highly variable within and between participants and were not normally distributed. S-P ratios exhibited a median of 2.0 and ranged from 0.0 to 23.0 (n = 67); 95% of data occurred between 2.3 and 4.3, and the 5% trimmed median was 2.7. The time-course evolution of S-P ratio data for each dosage regimen during the 24 h after METH administration are presented in Fig. 2.

Linear regression analysis of the relationship between

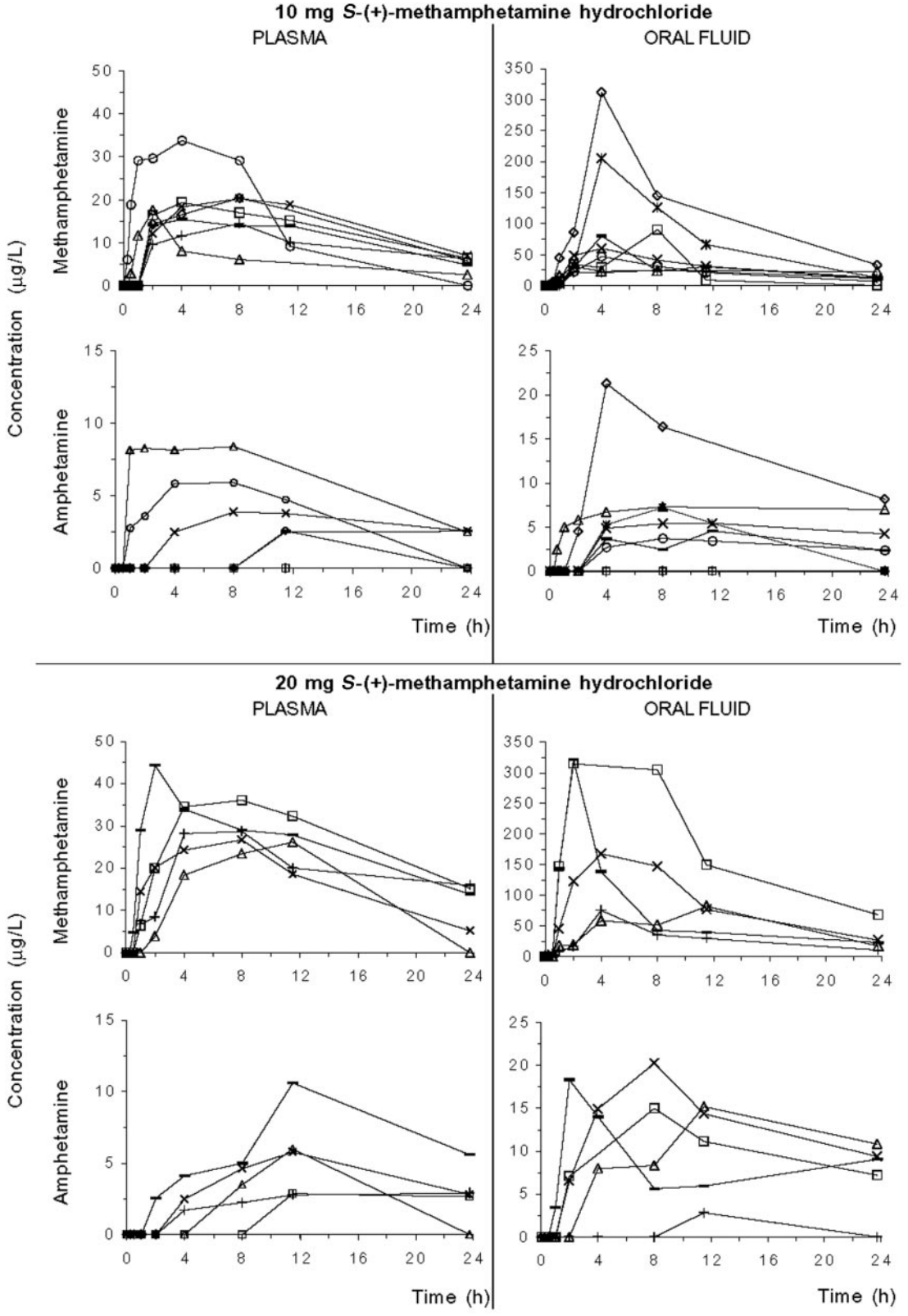

Fig. 1. Drug concentration profiles in plasma (*left*) and oral fluid (*right*) after administration of 10- and 20-mg sustained release *S*-(+)-methamphetamine hydrochloride.

All eight volunteers received the first low dose, but no plasma specimens were available for Z (*). Volunteers S (□), W (△), Y (×), AA (+), and BB (–; n = 5) received both the 10- and 20-mg doses. ◇, volunteer V; ○, volunteer X. Oral fluid samples were obtained after stimulation of expectoration with citric acid candy.

Table 2. Noncompartmental analysis of 24-h plasma and oral fluid AMP concentration data from session one after single 10- and 20-mg oral doses of sustained-release METH.[a]

	METH dose			
	10 mg (n = 8)[b,c]		20 mg (n = 5)	
AMP	Mean ± SD	Range	Mean ± SD	Range
Plasma				
t_{lag}, h	6.6 ± 5.3	1.0–12.0	7.4 ± 4.3	2.0–12.0
t_{max}, h	11.9 ± 6.9	8.0–24.0	14.3 ± 5.5	12.0–24.0
c_{max}, μg/L	4.7 ± 2.5	2.6–8.4	5.6 ± 3.2	2.9–10.6
Oral fluid				
t_{lag}, h	3.1 ± 1.5	0.5–4.0	4.1 ± 4.3	1.0–12.0
t_{max}, h	9.1 ± 3.0	4.0–12.0	8.2 ± 3.5	2.0–12.0
c_{max}, μg/L	8.6 ± 6.5	3.8–21.3	14.3 ± 6.1	2.8–20.2

[a]Citric acid candy was used to stimulate expectoration.

[b]After the 10-mg dose, plasma AMP concentrations were <2.5 μg/L in two volunteers and AMP was not detected in oral fluid specimens from three volunteers.

[c]No plasma samples were collected from volunteer Z.

oral fluid and plasma concentrations produced a R^2 value of 0.222 ($P = 0.005$).

COMPARISON OF THE DIFFERENT ORAL FLUID COLLECTION METHODS

Comparison of AUCs for oral fluid METH concentrations after the four low and four high sessions demonstrated a significant effect for dose ($P < 0.001$), device ($P = 0.006$), and subject ($P = 0.003$); there was no significant interaction between dose and device. AUCs for METH concentration in oral fluid collected with neutral cotton swabs (3416 ± 2309 and 6119 ± 2375 h·μg/L after a low and a high dose, respectively) were on average 1.47 (95% confidence interval, 0.75–2.91) times higher than those from citric acid-treated swabs (2388 ± 2029 and 3379 ± 1521 h·μg/L), which were on average 1.31 (95% confidence interval, 0.75–2.28) times higher than those after citric acid candy stimulation (1773 ± 1955 and 2496 ± 1609 h·μg/L). When oral fluid was collected with neutral Salivette devices, the AUCs for METH concentration averaged 1.92 (95% confidence interval, 1.06–3.51) times those of citric acid candy ($P < 0.05$). Over four doses, AUCs for oral fluid METH concentration were 3.06 (range, 2.12–4.43) times higher after the high dose than after the low METH dose ($P < 0.05$). Other METH pharmacokinetic results, obtained with noncompartmental analysis for the three different oral fluid collection methods, are summarized as Data Supplements available with the online version of this article at http://www.clinchem.org/content/vol49/issue1/.

ORAL FLUID pH

Both collection method ($P < 0.001$) and subject ($P < 0.001$) had significant effects on oral fluid sample pH. The pH of specimens collected with citric acid-treated cotton swabs (mean, 2.8 ± 0.3; range, 2.4–3.6; n = 130) was on average

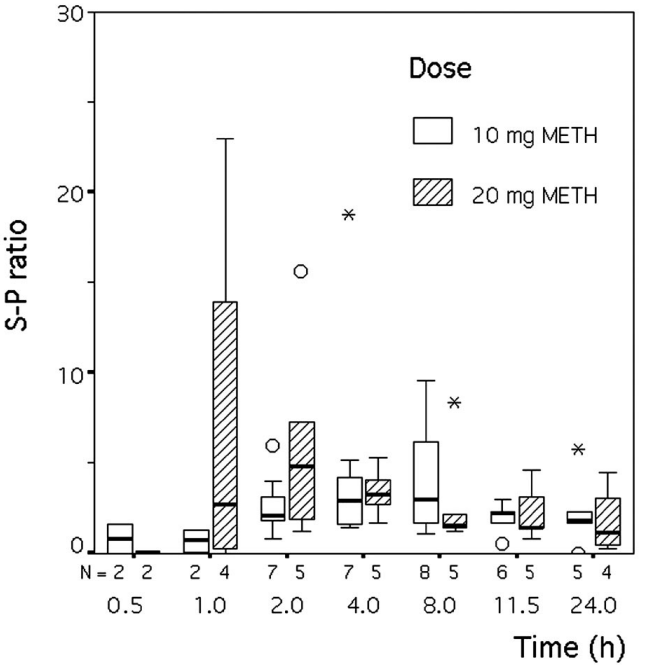

Fig. 2. Box plots showing S-P ratios after administration of 10- (□) and 20-mg (▨) sustained-release *S*-(+)-methamphetamine hydrochloride.

Data represent results from seven volunteers who received the first low dose and five who received both the 10- and 20-mg doses. The *boxes* represent the interquartile ranges of values and contain 50% of values. The *line across each box* indicates the median. The *error bars* indicate the highest and lowest values when outliers and extremes are excluded. ○ indicate outliers (values between 1.5 and 3 times the box length from the upper or lower edge of the box). ∗ indicate extreme values (values more than three box lengths from the upper or lower edge of the box). *N* denotes the number of cases in each box.

1.5 pH units lower than those collected after citric acid candy stimulation (mean, 4.3 ± 0.8; range, 3.4–7.2; n = 260). The latter yielded lower pH values (~1.7 pH units) than did oral fluid collected with the neutral cotton swab (mean pH, 6.0 ± 0.6; range, 4.2–7.2; n = 130).

WINDOWS OF DETECTION

Five of seven participants had plasma METH concentrations >2.5 μg/L 24 h after a single 10-mg dose (range, 4.8–6.9 μg/L); after a single 20-mg METH dose, four of five participants had METH concentrations of 5.2–15.9 μg/L at 24 h. Three of seven participants had plasma METH concentrations above the LOQ 24 h after four 10-mg METH doses (range, 4.3–6.6 μg/L); after four 20-mg doses, four of five participants had METH concentrations of 4.2–21.6 μg/L at 24 h.

In oral fluid obtained by citric acid candy-stimulated expectoration, METH was detected in all participants 24 h after a single 10- or 20-mg dose (range, 2.5–33.2 and 10.5–68.1 μg/L, respectively). After four low or high doses within 7 days, METH always was detected in neutral Salivette specimens collected at 24 h (range, 5.3–126.9 μg/L) and was present in citric acid-stimulated expectorations 36–76 h after the fourth dose (range, 2.7–7.3 μg/L).

In 2001, the Substance Abuse and Mental Health Services Administration (SAMHSA) proposed cutoffs for

METH and AMP confirmation in oral fluid. To be considered positive, the sample must contain ≥50 μg/L METH and an amount of AMP equal to or above the method's LOQ for AMP *(26)*. When we used these proposed cutoffs, METH was detected in our study in 0%, 0%, 13%, 50%, 29%, and 0% of the citric acid-stimulated oral fluid specimens collected 1, 2, 4, 8, 11.5, and 24 h after a single 10-mg METH dose, respectively. Similarly, after a single 20-mg METH dose, 20%, 60%, 80%, 60%, 60%, and 20% of samples were positive for METH exposure 1, 2, 4, 8, 11.5, and 24 h after drug administration, respectively. After four 10-mg METH doses within 7 days, 33% of participants had >50 μg/L METH and 2.5 μg/L AMP in their oral fluid collected with neutral Salivettes 24 h after drug administration; 80% were positive 24 h after four 20-mg doses. Forty-eight hours after the last of four 10- or 20-mg METH administrations, METH and AMP concentrations in oral fluid were below the proposed SAMHSA cutoffs.

PHARMACOLOGIC EFFECTS

There was a significant relationship between dose and diastolic blood pressure ($P = 0.003$) and dose and heart rate ($P = 0.032$; Fig. 3). When we performed pairwise comparisons of physiologic AUCs, the diastolic blood pressure (10.1%; $P = 0.003$) and heart rate (21.7%; $P = 0.036$) were significantly increased only when for the high dose compared with placebo. The AUCs for systolic blood pressure, skin and core temperature, blood oxygen saturation, and pupil diameter during drug treatment were not significantly different from those obtained during placebo treatment. Interestingly, participants' skin temperature decreased steadily for 2 h after drug administration, after which time temperatures increased toward baseline values.

Discussion

With oral administration of drugs, substantial buccal contamination of oral fluid can occur *(27)*. However, in the present study, there was an initial lag period before METH was detected, indicative of a lack of buccal contamination. This permitted the determination of appearance of METH and AMP in oral fluid.

When making a comparison of oral plasma METH pharmacokinetic parameters across different studies, it is important to verify the drug formulations used in these studies. Cook et al. *(16)* and Perez-Reyes et al. *(19)* used deuterated METH in a gelatin capsule for the determination of METH pharmacokinetics before and after repeated daily dosing with Desoxyn Gradumet, Shappell et al. *(18)* administered METH contained within a gelatin capsule, and Driscoll et al. *(21)* used oral liquid METH. In these four studies, METH formulations were not slow release, as in our study; thus, we expected differences in METH t_{max} and c_{max} values. In contrast, we expected METH elimination half-lives to be similar despite different absorption rates.

Mean plasma METH t_{max} values reported in our study after a single low dose ($t_{max} = 5.4 \pm 2.5$ h) did not differ significantly from those after the high dose ($t_{max} = 7.5 \pm 3.4$ h; overall mean $t_{max} = 6.3 \pm 3.0$ h) and were longer than those reported after administration of different oral METH formulations (2.9–3.6 h) *(16, 18, 21)*. Perez-Reyes et al. *(20)* used the same Desoxyn Gradumet formulation as in our study, but they reported a shorter mean METH t_{max} (3.0 h), possibly because of more frequent sampling. Mean plasma t_{max} values reported after vapor inhalation or intravenous administration reflect different absorption kinetics.

The mean plasma METH c_{max} values reported in our study after a single 10-mg dose (20.2 ± 6.4 μg/L) and 20-mg dose (32.4 ± 7.7 μg/L) were similar to those reported in other studies after oral administration of comparable doses: after administration of ~10 mg/70 kg of body weight, METH c_{max} was 20, 22, and 20.1 ± 7.0 μg/L, and after administration of 15 mg/70 kg of body weight, METH c_{max} was 39.5 ± 3.7 μg/L, regardless of the type of oral formulation *(16, 19, 21)*. Higher mean plasma METH c_{max} values were reported after METH vapor inhalation (47.1 ± 5.6 and 44.4 μg/L), reflecting faster absorption via the smoked route *(17, 20)*.

In our study, plasma METH half-lives were not dose dependent. METH half-lives averaged 9.3 ± 3.7 h after the low and 11.1 ± 7.2 h after the high dose, providing an overall mean half-life of 10.0 ± 5.2 h, in agreement with those reported previously (9.1 ± 2.2 h after ~10 mg/70 kg METH; 11–12 h, 11.8 ± 3.3, 13.1 ± 3.8, and 11.2 ± 3.7 h after ~15–18.5 mg/70 kg; 9.1 ± 4.0 h after 30 mg/70 kg; see Table 3) *(16–18, 20–22)*. Thus, as expected, the METH half-life was less influenced by drug formulation. As with plasma, the mean oral fluid METH elimination half-lives in our study (7.1 ± 2.3 and 8.1 ± 1.9 h after 10- and 20-mg doses of METH, respectively) were not significantly different between doses and averaged 7.6 ± 2.1 h. Oral fluid elimination half-lives were comparable to those in plasma.

Cook et al. *(16)* and Shappell et al. *(18)* fit their plasma METH data after oral administration of METH in a gelatin capsule to a one-compartment model with lag time and first-order kinetics. When we applied this model to data from our study, the mean absorption rate constant K_{01} ($K_{01} = 0.93 \pm 0.61$ h^{-1}; n = 13) was intermediate between the K_{01} described by Shappell et al. ($K_{01} = 0.82 \pm 0.45$ h^{-1}) *(18)* and Cook et al. ($K_{01} = 1.36 \pm 0.15$ h^{-1}; n = 24) *(16)* despite differences in drug formulation. The mean plasma elimination rate constant of the slow-release formulation ($K_{10} = 0.085 \pm 0.067$ h^{-1}; n = 13) was similar to the K_{10} reported by Cook et al. ($K_{10} = 0.074 \pm 0.004$ h^{-1}; n = 24) and Shappell et al. ($K_{10} = 0.097 \pm 0.063$ h^{-1}; n = 10) *(16, 18)*. The relative constancy of estimated half-lives observed in all studies after administration of different doses via a variety of routes is also supportive of first-order elimination kinetics for METH *(16–18, 20–22)*.

In our study, plasma AMP concentrations were always lower than METH concentrations, with ratios ranging from 16% to 37%. A comparison of 24-h AUCs for plasma AMP after low (mean, 40 ± 33 h · μg/L) and high doses (mean, 81 ± 44 h · μg/L; n = 5) suggested a dose–

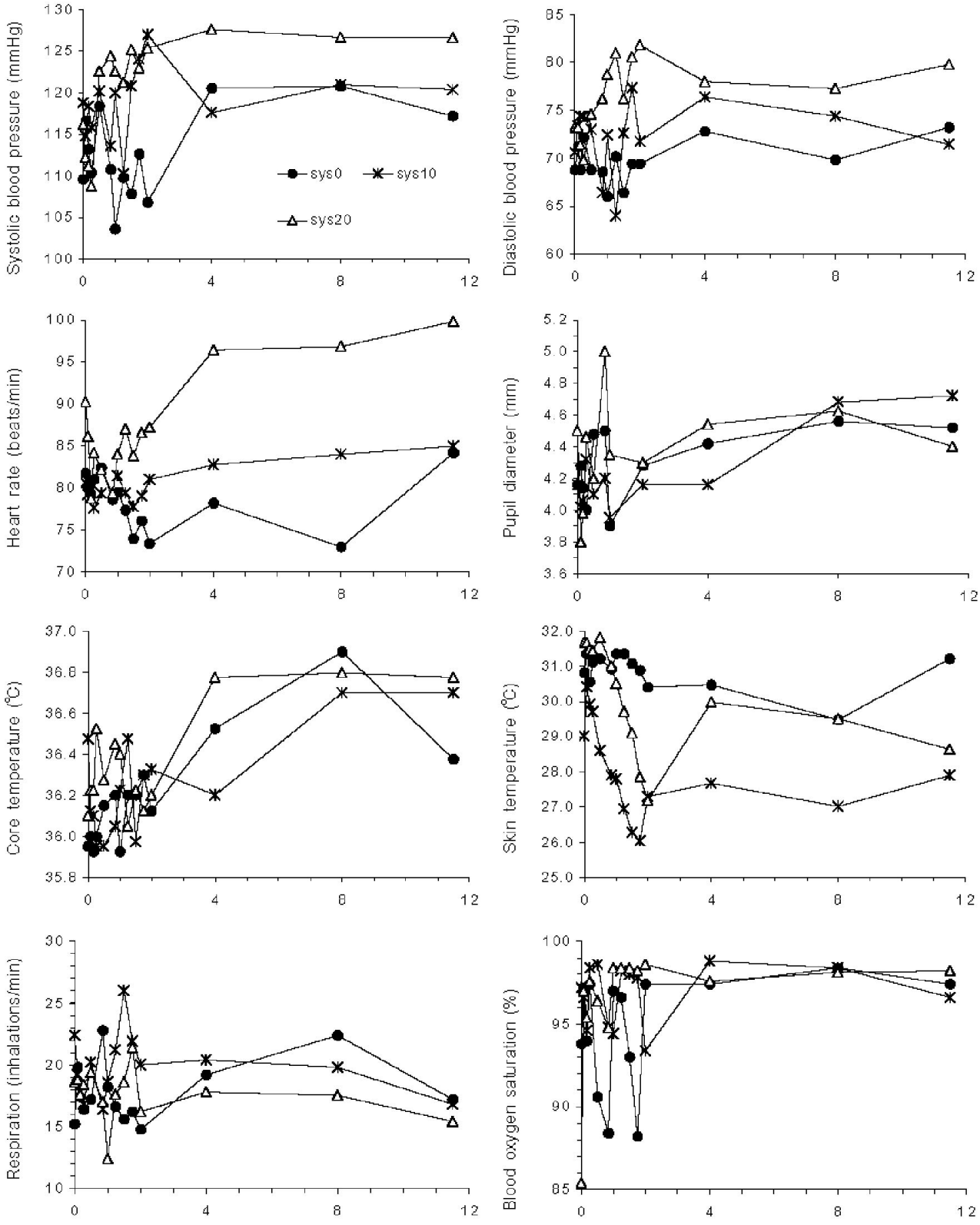

Fig. 3. Mean (n = 5) changes in physiologic effects as a function of time after administration of placebo (○) or 10 (×) or 20 mg (△) of sustained-release methamphetamine hydrochloride.

In pairwise comparisons, only increases in diastolic blood pressure and heart rate for 20-mg sustained-release METH were significant compared with placebo, based on the 12-h AUCs.

Table 3. Plasma METH concentrations reported in controlled drug administration studies.

				Mean ± SD		
Authors[a]	METH dose[b]	Formulation	n	t_{max}, h	c_{max}, μ g/L	$t_{1/2}$, h
Driscoll et al. *(21)*	12.5 mg	Liquid	10	3.0	20.0	NA[c]
Perez-Reyes et al. *(20)*	22 mg	Vapor inhalation	6	2.5 ± 0.5[d]	47.1 ± 5.6	11–12 (8–17)[e]
Cook et al. *(17)*[e]	21.8 mg	Vapor inhalation	6	2.0[d,e]	44.4[e]	11.8 ± 3.3
	15.5 mg	Intravenous	6	4 min[e]	101[e]	13.1 ± 3.8
Shappell et al. *(18)*	30 mg/70 kg	Capsule	10	3.6 ± 0.6	94.1 ± 70.9	9.1 ± 4.0
Perez-Reyes et al. *(19)*	0.125 mg/kg	Capsule	6	3.0 ± 0.9	22.0	NA
Cook et al. *(16)*[f]	0.125 mg/kg	Capsule	12	3.3 ± 0.9	20.1 ± 7.0	9.1 ± 2.2
	0.250 mg/kg		12	2.9 ± 0.5	39.5 ± 3.7	11.2 ± 3.7
Current study	10 mg	Desoxyn Gradumet	8	5.4 ± 2.5	20.2 ± 6.4	9.3 ± 3.7
	20 mg		5	7.5 ± 3.4	32.4 ± 7.7	11.1 ± 7.2

[a] The pharmacokinetic data from the METH inhalation study were reported twice *(17, 21)*. The pharmacokinetic data reported by Perez-Reyes et al. *(19)* are a subset of the data reported by Cook et al. *(16)*.

[b] Methamphetamine · HCl was used in all studies.

[c] NA, data not available.

[d] After methamphetamine · HCl vapor inhalation, METH appeared rapidly in plasma; thereafter, plasma concentrations remained at a plateau for at least 2 h.

[e] Data were obtained from Dr. C.E. Cook (personal communication). Values in parentheses indicate the range.

[f] Perez-Reyes et al. *(19)* and Cook et al. *(16)* used d_3-methamphetamine · HCl for determination of pharmacokinetic parameters.

response relationship; AUCs for 10-mg doses were on average 2.3 ± 1.5 times lower. On a molar basis, 24-h AUCs for plasma AMP averaged 21% ± 25% of those observed for the parent drug. Cook et al. *(16)* demonstrated a plasma AMP dose–concentration relationship and reported AMP AUCs that were 30% of those observed for the parent drug after oral METH administration and 14–17% after METH vapor inhalation or intravenous administration *(17)*. Interestingly, whereas our mean plasma AMP c_{max} values (4.7 ± 2.5 and 5.6 ± 3.2 μg/L after the low and high dose respectively; Table 4) were similar to those of Cook et al. *(16)* for a different oral formulation (1.6 ± 0.6 and 4.0 ± 1.5 μg/L after the low and high doses, respectively), our 24-h AUCs for AMP tended to be lower than those reported by Cook et al. [98 ± 67 and 224 ± 155 h · μg/L after the first low and high dose, respectively *(16)*]. This discrepancy can be explained by the fact that the lower method LOQ used by Cook et al. (1 μg/L) and higher number of sampling points permitted earlier and longer detection of low quantities of AMP, thus increasing the AMP AUC.

Peak plasma AMP concentrations always occurred after maximum METH concentrations, as reflected in their respective mean t_{max} values (overall AMP t_{max} = 13.1 ± 6.0 h vs overall METH t_{max} = 6.3 ± 3.0 h). Perez-Reyes et al. *(20)* and Cook and coworkers *(16, 17)* reported similar AMP t_{max} values after METH intake or inhalation. The reported mean AMP t_{max} value after intravenous administration from Cook et al. reflected a plateau value: AMP concentrations reached near-maximum values 10 h after drug administration, and thereafter they remained at a plateau for ~14 h *(17)*.

The usefulness of oral fluid drug concentrations in lieu of plasma measurements for TDM depends mainly on the constancy of the S-P ratio *(15)*. In our study, the within- and between-subject variability was considerable. This predicted

Table 4. Plasma AMP concentrations reported in various controlled drug administration studies.

				Mean ± SD	
Authors[a]	METH dose[b]	Formulation	n	t_{max}, h	c_{max}, μg/L
Perez-Reyes et al. *(20)*	22 mg	Vapor inhalation[c]	6	10–24	3–6
Cook et al. *(17)*	21.8 mg	Vapor inhalation[c]	6	12 ± 5.6	4.2 ± 1.4
	15.5 mg	Intravenous	6	17 ± 8.1	4.0 ± 1.5
Cook et al. *(16)*[d]	0.125 mg/kg	Capsule	10	11.7 ± 3.5	1.6 ± 0.6
	0.250 mg/kg		9		4.0 ± 1.5
Current study[e]	10 mg	Desoxyn Gradumet	5	11.9 ± 6.9	4.7 ± 2.5
	20 mg		5	14.3 ± 5.5	5.6 ± 3.2

[a] The pharmacokinetic data reported by Perez-Reyes et al. *(20)* and Cook et al. *(17)* originated from the same clinical study.

[b] Methamphetamine · HCl was used in all studies.

[c] After methamphetamine · HCl vapor inhalation, AMP appeared in plasma rapidly and remained at a plateau for at least 2 h.

[d] Cook et al. used d_3-methamphetamine · HCl for the determination of pharmacokinetic parameters. AMP t_{max} values were reported as the overall mean (n = 19).

[e] Plasma AMP concentrations were generally too low to allow for further pharmacokinetic analysis. After a single oral 10-mg dose of METH, AMP was not detected in two volunteers. No plasma data were available for a third volunteer.

a poor correlation between plasma and oral fluid METH concentrations, which was confirmed by a low R^2 value of 0.222. Therefore, oral fluid concentrations could not be reliably used to predict plasma concentrations. The theoretical METH S-P ratio, as calculated by the Henderson–Hasselbalch equation, is 4.0 *(28)*. Cook et al. *(16)* reported a ratio averaging 7.8 ± 6.2 with a R^2 value of 0.498, indicating that oral fluid measurements cannot readily be used to accurately predict corresponding plasma concentrations. Our median S-P ratio (2.0) was not substantially different from the mean S-P ratio reported by Cook et al. *(16)* after oral METH administration because of the high variability in this parameter. Within a single individual, there appeared to be no increasing or decreasing trend in S-P ratios within 24 h after drug administration.

Higher concentrations of METH and AMP in oral fluid compared with plasma could be attributed to active specific salivary transport *(29)* or to ion trapping *(30)*. In the ion-trapping model, the pH difference between blood (pH 7.4) and saliva (pH ~6.7) provides the driving force for diffusion and partitioning of basic molecules such as METH (pK_a = 10.1) and AMP (pK_a = 10.0) *(31)* into saliva.

METH concentrations were higher in oral fluid specimens collected with cotton swabs than in citric acid candy-stimulated expectorations. In comparison, oral fluid volumes collected with Salivette were smaller than those obtained with citric acid candy. Thus, we found low analyte concentrations in high-volume specimens, suggesting a dilution effect. The dilution might be attributable to a limited METH diffusion rate, when METH lipid solubility is insufficient to permit rapid and complete plasma–saliva equilibration when salivary secretion is stimulated further *(15, 30)*.

The windows of detection and the detection rates (number of positive specimens divided by the total number of specimens collected in that time period) were investigated for up to 24 h after drug administration because of study design. After a single low or high dose, plasma METH was >2.5 μg/L for up to 24 h in 9 of 12 participants (mean, 7.3 ± 5.5 μg/L at 24 h); the 24-h detection rate was 54% (n = 130) based only on the presence of METH. All citric acid candy-stimulated oral fluid specimens collected at 24 h after drug administration (n = 13) contained >2.5 μg/L METH (mean, 18.8 ± 18.0 μg/L); AMP concentrations were also ≥2.5 μg/L in 11 of these samples. The METH detection rate in oral fluid for all specimens collected within 24 h of drug administration was 60% (n = 130) when we used the 2.5 μg/L METH cutoff; when we used a 2.5 μg/L cutoff for both METH and AMP, the 24-h detection rate was 23% (n = 130). When we used the SAMHSA-proposed METH and AMP cutoffs (50 and 2.5 μg/L, respectively), only 1 of 13 individuals tested positive 24 h after a single METH dose; 23 of 130 samples within the 24-h time period after dosing had METH and AMP concentrations >50 and >2.5 μg/L, respectively (detection rate of 18%).

These detection rates show that after a single METH dose, oral fluid is equivalent to plasma for detecting METH abuse within 24 h. However, it is expected, based on plasma and oral fluid concentrations at 24 h, that oral fluid will be superior to plasma for detecting METH abuse beyond 24 h, depending on the cutoff concentrations. The data also demonstrate that 24-h oral fluid detection rates are reduced when ≥2.5 μg/L AMP is required to be present in expectorations.

After four 10- or 20-mg doses, METH was >2.5 μg/L in citric candy-stimulated oral fluid specimens for 36–72 h (mean, 58.3 ± 14.5 h); all of these samples had AMP concentrations >2.5 μg/L. After four low or high METH doses within 7 days, with the SAMHSA-proposed cutoffs, 8 of 13 individuals were positive for METH 24 h post dose when sampled with the neutral Salivette; 48 h after the last dose, all were negative. Suzuki et al. *(17)* reported detectable METH in oral fluid in two cases for 48 h after self-administration of drug. The LOD of the method used in their study was 20 pg on column; the amount of oral fluid analyzed was not defined. These data suggest that the detection window for METH in oral fluid can be extended considerably when the METH cutoff is reduced from 50 μg/L to 2.5 μg/L.

Urine is the most commonly used matrix for detecting drug abuse in workplace, criminal justice, and driving under the influence programs. Urine METH and AMP concentrations in our study were ≥2.5 μg/L early after a single 10-mg sustained-release dose (2.8 ± 1.6 h) with a 24-h detection rate ≥92% (n = 31), a considerably higher rate than those found in our plasma and oral fluid specimens *(32)*. Urine METH and AMP concentrations remained quantifiable (≥2.5 μg/L) for extended periods of time after multiple doses (range, 46–169 h; mean, 110.7 ± 34.4 h). In general, urine drug concentrations were also considerably higher than the corresponding oral fluid or plasma concentrations, as reported also by Cook et al. *(16)*, facilitating prolonged drug detection.

Analysis of pharmacodynamic data revealed a modest but significant (P <0.036) increase in diastolic blood pressure (10%) and heart rate (22%) after a single 20-mg sustained-release METH · HCl dose compared with placebo. Similar cardiovascular effects were reported previously by Perez-Reyes et al. *(19)*, who used the same formulation and route of METH administration, and by Shappell et al. *(18)* after a single 30 mg/70 kg oral dose. In addition, the cardiovascular effects were demonstrated in squirrel monkeys *(7)* and in freely moving, nonanesthetized rats after METH administration *(33)*. The initial drop in skin temperature also was observed in rats after a single intravenous injection of 3 mg/kg METH *(33)*. Arora et al. *(33)* demonstrated that after a METH injection, skin temperature dropped within minutes and increased again over ~2 h to remain increased for the next 8 h. Caution should be applied when interpreting changes in body and/or skin temperatures as these are complex, highly dependent on the environmental temperature and dependent on the dose of METH administered.

Data from this study suggest that the disposition of METH in oral fluid appears to be dose related; however, high intra- and intersubject variability limits the usefulness of single oral fluid measurements to predict concurrent plasma concentrations for TDM. On the other hand, oral fluid is a viable biological matrix to monitor METH use in workplace, criminal justice, and driving under the influence testing programs. Compared with urine, oral fluid monitoring offers a more practical sampling method with improved adulteration control; however, a lower analyte concentration and a shorter window of detection may be less advantageous for METH monitoring with this biological matrix.

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