ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 2, pp. 237–248, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00076-7

Generation of Stable Test Atmospheres of Cocaine Base and Its Pyrolyzate, Methylecgonidine, and Demonstration of Their Biological Activity

RONALD W. WOOD,[1] JOHN F. GRAEFE, CHING PING FANG, JALIL SHOJAIE, LUNG CHI CHEN AND JOYCE WILLETTS

Nelson Institute of Environmental Medicine, NYU Medical Center, Longmeadow Road, Tuxedo, NY 10987

Received 7 July 1995; Revised 11 December 1995; Accepted 27 December 1995

WOOD, R. W., J. F. GRAEFE, C. P. FANG, J. SHOJAIE, L. C. CHEN AND J. WILLETTS. *Generation of stable test atmospheres of cocaine base and its pyrolyzate, methylecgonidine, and demonstration of their biological activity.* PHARMACOL BIOCHEM BEHAV **55**(2) 237–248, 1996.—Generating controlled test atmospheres of known chemical identity and airborne concentration upon demand is a significant technical obstacle that limits the scope and repeatability of studies of inhaled substances. We addressed this problem as applied to the generation of atmospheres that result from heating crack cocaine, which include both cocaine and its pyrolyzate methylecgonidine (MEG). A condensation aerosol generator was used to generate atmospheres comprised of monodisperse particles of cocaine, MEG, or mixtures of both that are of submicron size suitable for deposition in the alveolar region of primates. Compressed air seeded with nanometer-size sodium chloride particles was passed through a constant depth of molten cocaine or MEG in a bead bed, reheated, and condensed to an aerosol within an annulus of cold air. To achieve control of a mixture of both compounds, MEG was condensed onto cocaine particles in a separate coating step. On-line analytical instruments provided verification of airborne concentration, estimates of particle size, and dispersion as well as chemical identity. Specific airway conductance (SGaw), heart rate, and rectal and skin temperatures were measured in squirrel monkeys breathing atmospheres containing condensation aerosols of cocaine or MEG free base. SGaw was reduced after inhalation of either base, and both induced temperature and cardiovascular changes, demonstrating that the aerosols so generated had biological activity.

Crack Condensation aerosols Cocaine Methylecgonidine Anhydroecgonine methyl ester Aerosol Body temperature Bradycardia Bronchoconstriction Cardiovascular Heart rate Squirrel monkeys

"CRACK" is a form of cocaine base self-administered by smoking. As the cocaine travels away from the flame and cools, the vapor condenses forming smoke, a condensation aerosol comprised of cocaine base droplets and associated pyrolysis products (22). One obstacle limiting the growth of an experimental literature on this substance abuse practice is the challenge offered by generating a defined and reproducible test atmosphere upon demand and appropriate for the test species. Boni et al. (3) developed a single bolus delivery system for use with rodents that produced exposure-related changes in cardiovascular function. This system requires manual delivery by the investigator, and could not provide information about the chemical and physical properties of each individual exposure. These investigators demonstrated 69% deposition of cocaine in the upper respiratory passages of the rat. Alveolar deposition comparably effective to that observed in humans might not be attained in other laboratory animal species unless care is taken to control the particle size distribution of the aerosol (2,14). We recently studied the hotwire technique with which cocaine base is volatilized from a nichrome wire (6). This procedure tends to produce a polydisperse particle size distribution with a large mass median aerodynamic diameter, and lower levels of pyrolysis than that observed during simulated crack smoking in a model crack pipe (22).

In order to enable a better understanding of the acute and chronic effects of cocaine base smoking, as well as of the

[1] To whom requests for reprints should be addressed: Box 668, University of Rochester Medical Center, Rochester, NY 14642.

physical and chemical processes involved in smoking per se, aerosol science technologies were applied to the task of generating and characterizing cocaine base test atmospheres. Because the size of each particle is an important determinant of the location of airway deposition (2,14), rapidity of onset, and location of acute and chronic effects, control of this parameter may be required to achieve mass deposition in the alveolar region comparable to that attained by humans. The technologies required to control particle size also enable stable operation of generation systems for hours. Because pyrolysis may occur with the application of heat to alkaloids, it is important to be able to generate pure atmospheres or known mixtures of the parent compound and its pyrolyzates and to verify that the desired test atmosphere has been achieved.

After pursuing other strategies, we settled upon a condensation aerosol generator similar to those designed for the calibration of optical instruments (11,13,15,18). These early systems had several design features that guided our current effort: 1) the median diameter of a condensation aerosol particle size distribution can be made smaller and more uniform by seeding the vapor with condensation nuclei, nanometer size particles with a very large surface area in comparison to their mass; 2) a boiler holding the material to be vaporized, through which bubbles the condensation nuclei in the carrier gas; 3) a reheat zone to insure that all of the material is in the vapor phase before aerosol formation commences; and 4) a condensation zone, a downward-flowing chimney in which cooling leads to aerosol formation. The original designs were optimized for the present purpose, and supplemented with analytical instrumentation for continuous or near continuous monitoring of airborne mass concentrations, the mass median optical diameter and geometric standard deviation of the aerosol diameter distribution, and the chemical constituents of the generated atmosphere. The present system permits the stable generation and independent control of the airborne concentration of cocaine and its principal pyrolysis product anhydroecgonine methylester (methylecgonidine; MEG). The particle size distributions generated are stable and of low variability, suitable for achieving high alveolar deposition. Their suitability for use in laboratory studies of the effects of cocaine and MEG is demonstrated here in exposures to squirrel monkeys.

METHOD

The cocaine aerosol generation and delivery system (Fig. 1) consists of an aerosol generator, a dual acting electropneumatic ball valve assembly for routing the aerosol or clean air, a dilution system, and analytical equipment for monitoring airborne concentration and particle size. The system as described is the final stable operating configuration; the generator configuration and some operating conditions were varied for experimental purposes described herein.

Condensation Nuclei

To provide condensation nuclei, a ceramic boat was filled with sodium chloride and placed inside an alumina ceramic tube (McDanel Refractory Co., Beaver Falls, PA). The tube was covered at each end with a Viton rubber gasket and stainless steel plate assembly with threaded fittings. The ceramic tube was placed inside a tube oven (Lindberg Model 55035, General Signal, Watertown, WI) at 725°C. The alumina tube was purged with dry air at 5 l/min regulated with a mass flow controller (Model RO-28/FC280SAKZ, Tylan General Corp, Torrance, CA). The effluent passed through a short finned radiator, which reduced the temperature to approximately 150°C. This process produced submicron particles of very low mass, but very high number concentrations, $10^9/cm^3$; these primary particles coagulate rapidly, reducing the concentration within 30 s to a concentration still greater than $10^7/cm^3$. Electron microscopy revealed that the vast majority of these particles were less than 0.1 μm in diameter. Because the resultant cocaine particles are approximately 1 μm in diameter, the condensation nuclei represent less than 0.1% of the delivered mass, calculated from the relative particle volumes. The number of condensation nuclei could be reduced while maintaining constant flow rates by diverting a portion of this stream through a HEPA filter. This condensation nucleus flow was then heated in a 2 m long coil of 3/16″ OD copper tubing at 160 to 175°C (preheat zone) before it passed to the generator.

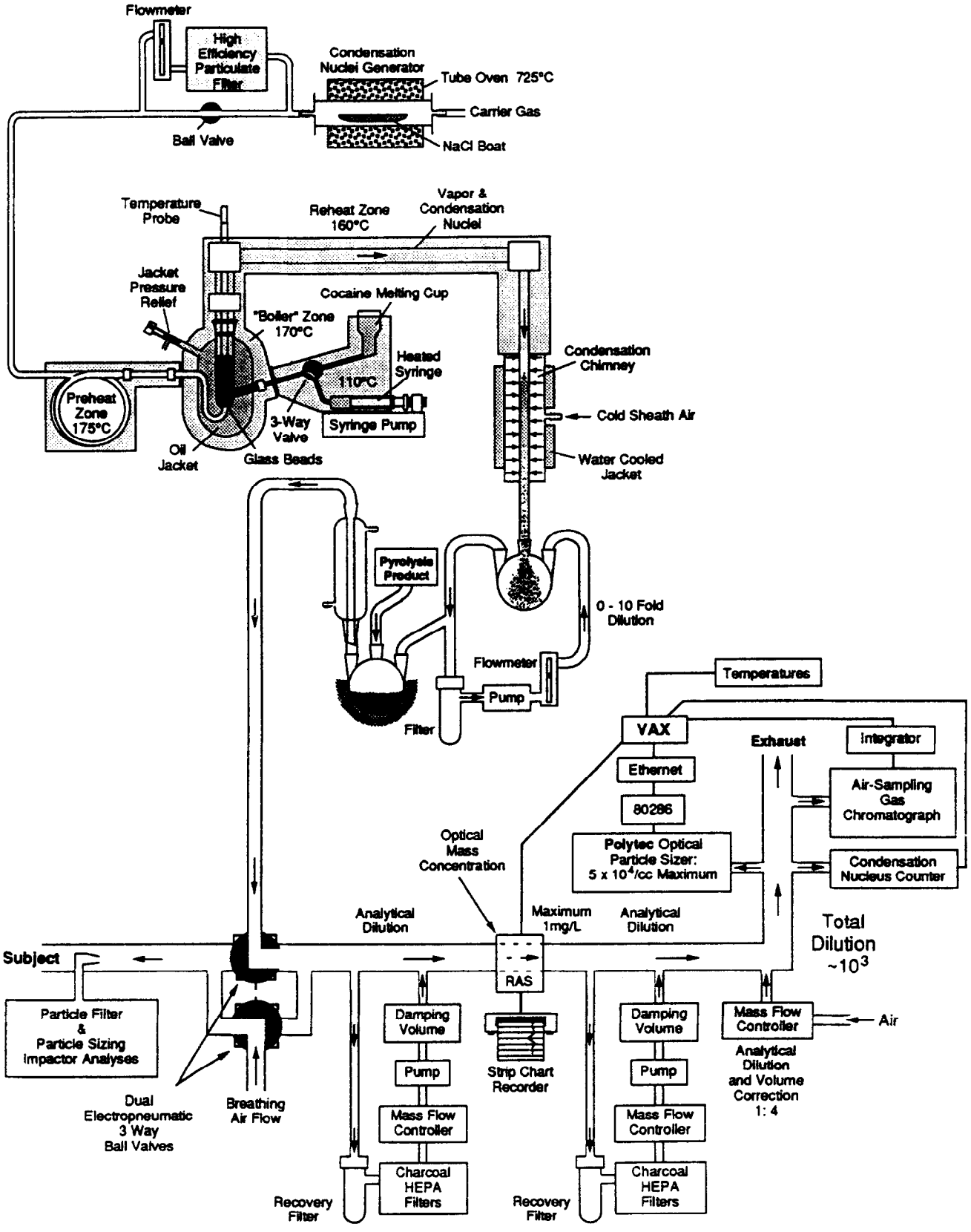
Condensation Aerosol Generator

The generator system is illustrated in Fig. 1. The condensation nucleus flow entered the bottom of an oil-jacketed hot finger (boiler) filled with 3 mm diameter glass beads resting on a disk of stainless steel screening (Ace Glass, Inc., Drawing #V-1574, Vineland, NJ). The boiler was fit within a cylindrical heating mantle (GlasCol TM570, Terre Haute, IN). The residual volume of the finger was 10–15 ml, and the beads provided added surface area on which vapor saturation can occur. The NaCl seed–cocaine vapor mixture then flowed into a reheating zone to assure complete volatilization and, thus, a uniform particle size distribution after condensation in the downward-flowing chimney. The reheating zone was fabricated from 0.75″ stainless steel tubing and compression fittings; the horizontal section was approximately 35 cm long. Temperatures in the preheat, reheat, and boiler zones were regulated by computerized temperature controllers (Model CN9122, Omega Engineering, Inc., Stamford, CT) and copper/constantan thermocouples that operated silicone or fiberglass heat tapes (Thermolyne, Corp, Dubuque, IA), or heating mantles. Areas heated with heat tape and exposed hot surfaces were wrapped in aluminum foil. Air temperature was continuously monitored in the reheat zone, as were temperatures elsewhere (Models DS-41-TC-A, DSS-670T-A Omega Engineering).

The condensation zone consisted of a porous (10 μm pore)

FIG. 1. Cocaine aerosol generation system. Condensation nuclei are generated in a tube oven, preheated and passed through cocaine held in a reservoir or "boiler." The level of cocaine is maintained by constant addition of molten cocaine using a heated syringe pump. The cocaine vapor and nuclei are reheated to ensure that there is no premature particle formation, and then passed into a downward-flowing condensation chimney in which the vapor is kept off the interior walls with annular sheath air. Dilution may be performed at this point to adjust the concentration before delivery to an animal. If MEG is to be coadministered, the aerosol can be passed through a heated vessel with the pyrolyzate and then cooled to coat the pyrolyzate on the viscous cocaine droplet. MEG may also be administered alone by placing it in the boiler. The aerosol then passes through a valve to the animal, or to the analytical path. After a single stage of dilution, it passes through a nephelometer (RAS) which generates a voltage proportional to airborne particle mass. After two more dilution steps, the atmosphere is passed to other analytical equipment, including an optical particle sizing device, an optical particle counter, and a gas chromatograph. Information from these various instruments is logged and displayed on computer systems.

COCAINE AEROSOL GENERATOR



1/2'' i.d. stainless tube (inertial gas sampling filter #7610-1/2-6-10, Mott Metallurgical Corp, Farmington, CT) enclosed in a pressurized cylindrical case. The case was wrapped with copper tubing and chilled with cold running water flowing in an upward direction through the coil. Compressed air introduced into the chilled case at 0.5 l/min formed a cool annulus of sheath air that prevented cocaine vapor from condensing on the walls and forming crystals that extended dendritically into the center of the condenser tube. Sheath air minimized wall loss and stabilized particle size and concentration, despite the added volume of diluent gas.

Syringe Addition Zone

A continuous feed system was devised to maintain a stable liquid level in the boiler. Cocaine was melted in a stainless steel cup attached to a stainless three-way valve with Teflon seats and washers (1/4'' i.d., Hoke 7165G4Y), and was drawn into a 20 or 50 ml stainless steel syringe (Harvard Apparatus, Inc., South Natick, MA) fitted with alkaloid-resistant perfluoroelastomer O-rings (KALREZ, Dupont, Wilmington, DE) using a large infusion-withdrawal syringe pump (Harvard Apparatus, Inc.). The O-rings were lubricated occasionally with a drop of polyphenylether oil, a high molecular weight oil with extremely low vapor pressure. After purging bubbles from the syringe, the valve position was changed so that the syringe delivered cocaine to the reservoir through 2 mm i.d. stainless hypodermic tubing and a septum into a glass line leading through the boiler's oil jacket to the lower portion of the hotfinger. The stainless steel tubing was jacketed in copper tubing for even heat transfer. Thermocouples were attached in several locations to detect hot or cold spots. The syringe, valve, and tubing assembly were wrapped with separate heating tapes and aluminum foil and each was regulated with a variable transformer. The entire syringe addition zone was maintained near 110°C for cocaine delivery. Pure MEG atmospheres were generated in the same way, except the syringe was held at room temperature.

Aerosol Delivery and Characterization System

The generated aerosol can be diluted to establish the desired exposure concentrations using a recirculating bellows pump and filtration system (see below). After the first stage of dilution, MEG can be coated on the cocaine droplets by first passing the droplets through MEG vapor in a three-neck flask; the MEG vapor condenses on the surface of the cocaine droplets upon cooling in a water-cooled condenser on the outlet of the coating flask. The aerosol was then ducted to the input of an electropneumatic dual three-way 0.5'' i.d. stainless steel ball valve with Teflon packing (Whitey/Swagelock, Highland Heights, OH). The large internal diameter of these valves was necessary to eliminate clogging and was the minimum internal diameter of the entire system. These two valves were plumbed to permit the diversion of aerosol flow from the analytical systems to the animal, or to filters or impactors used to characterize the atmosphere in the breathing zone of the test animal. At the conclusion of the timed delivery, clean air was restored to the animal; during delivery to the animal, this clean atmosphere passed through the analytical system, rezeroing it. When a cocaine test atmosphere was not being delivered to an animal, most (~93%) of the test atmosphere was drawn off and captured for recycling on a cartridge filter (Preflow 200/12631, Gelman, Ann Arbor, MI) in the first stage of analytical dilution. Once particle free, the air was drawn through a charcoal bed, a high-efficiency particulate (HEPA) filter and a mass-flow controller with Kalrez seals by an aluminum bellows pump with Teflon diaphragms (Models N022ATP, N035.1.2.ATP, KNF Neuberger, Princeton, NJ); it then passes through a 3.785 L damping volume and reenters the system. This maintained a constant volumetric flow while reducing the airborne concentration of the cocaine test atmosphere. In conjunction with mass-flow controllers regulating the carrier gas and subsequent dilution stages, this arrangement maintained steady dilution ratios throughout the system once the system is closed and adjusted using a calibrated bubblemeter (Gilibrator Model 800266, Gilian Instrument Corp, W. Caldwell, NJ).

Optical Measurement of Airborne Mass

After the first stage of analytical dilution, the airborne particle mass concentration was monitored optically with a rapidly responding nephelometer (Realtime Aerosol Sensor [RAS2], MIE Inc., Bedford, MA). Placed between the first two stages of analytical dilution, it provided immediate feedback for adjusting the exposure concentration. If the measure was unstable, there was either a problem with the generator or the preceding filter was clogged; the filter flow was monitored by checking the pressure drop across the filter on an aneroid pressure gauge (Dwyer Magnehelic, Michigan City, IN). If the nephelometer and the particle size were stable, the system was operating properly, because failure to produce vapor or condensation nuclei in appropriate amounts resulted in a change in particle diameter. If the concentrations downstream of the nephelometer were unstable, the second or third stages of dilution had a leak or clogged filter; this did not affect particle sizing, as long as the concentrations did not exceed the instrument's upper operating limits for particle number. The analysate flow was diluted twice more before sampling (approximately a 1000-fold total dilution) at 5 l/min through the optical head of the particle size analyzer (described below).

Optical Measurement of Particle Size Distributions

The Polytec HC2015 optical particle size analyzer (Polytec GmBH, Waldbronn, Germany) measures particles in situ at number concentrations up to 2.5×10^4 particles/cm^3 by focussing a beam of white light at a point in space where light scattering will occur as a single particle passes through a cubic measuring volume that is 110 μm on each side. Despite the intense light source, the particles are not subjected to further heat because the optical head is water cooled. The small measurement volume permits the measurement of high concentration atmospheres. The upper limit for accurate measurement of particles is a result of the likelihood of two particles being present in the measurement volume simultaneously. We demonstrated that this upper limit was 2.5×10^4 particles/cm^3. The instrument software assumes a uniform velocity profile across the diameter of the aerosol flow, but the particle velocity profile was parabolic under these laminar flow conditions. The actual velocity in the measuring volume was twice the assumption, and the instrument's estimation of number concentration reported here was twice the actual concentration. This was confirmed with an independent estimate using a condensation particle counter (CPC Model 3020, TSI Inc, St. Paul, MN).

The Polytec was controlled by a computer (Intel 80286 processor: Model 212LP, Digital Equipment Co., Marlboro,

MA) to periodically sample and compute the measures of particle size and uniformity using customized software (Kevin Horton, UK Atomic Energy Establishment Technology Center at Winfrith, Dorset, UK). We integrated this with a file server on our central VAX computer system (Digital Equipment Corporation) so the data were available for display cumulatively in real time (RS/1, BBN Software Products, Cambridge, MA).

Measurement of Particle Size Distributions by Inertial Impaction

A cascade impactor classifies particles by their aerodynamic size (7,19); it is used as a primary standard for determining particle size, and relies on the inertial impaction of particles on surfaces. The Mercer impactor used here (Model 02-170, Intox Products, Albuquerque, NM) consists of seven impaction stages, and one backup filter. The cutoff diameters for these stages are 8.1, 4, 2.1, 0.98, 0.5, 0.27, and 0.15 μm at a sampling flow of 5 l/min. Stage samples were promptly put into solution and held in a closed container for no more than 24 h before injection on the gas chromatograph. Similar procedures using filters and charcoal tubes were used for the measurement of airborne mass concentrations (22). The analytical chemistry of cocaine and MEG, including synthesis procedures, are described in detail elsewhere (22); significant amounts of cocaine and MEG were recovered routinely from filters and internal surfaces of the generator with methanol and acetone, and were recycled after purification.

Subjects

Three adult male squirrel monkeys (61, 63, and 1034) weighing 0.8–1.1 kg were used in these studies. They were housed individually in a room maintained at 26–27°C and 40% relative humidity. Room lights came on at 0600 and went off at 1800 h. During experimental sessions, monkeys were removed from their home cage and placed in restraining chairs (model XPL-512-SPC, Plas-Labs, Lansing, MI). Experimental sessions were of 80–120 min duration and were conducted no more than two times per week. After sessions, monkeys were returned to their home cages. The monkeys were allowed free access to water in their home cages and were fed Purina Monkey Chow daily, supplemented with fresh fruit and vegetables.

Physiological Procedures

After being seated in the restraining chairs, monkeys were fitted with sterile gold or platinum subdermal needle electrodes (model E2; Grass Instrument Co., Quincy, MA) placed subcutaneously in the left and right chest and left and right inner thigh to record electrocardiograms (ECGs). Leads were attached to an ECG amplifier/coupler (model S75-11; Coulborn Instruments, Columbus, OH) and tachometer (Model S77-26; Coulborn Instruments); the ECG wave (usually lead I) was visualized on an oscilloscope (Type 453; Tektronix, Portland, OR). A rectal thermocouple (Omega Engineering, Inc., Stamford, CT) was inserted to a depth of 10 cm to record core body temperature. Skin temperature was also recorded via a thermocouple taped to the monkey's foot. After preparation, chaired animals were placed inside a 31-liter whole-body plethysmograph with inside dimensions of 51.4 cm high × 26.4 cm wide × 22.9 cm deep. During most sessions, the internal temperature of the box was maintained at 29–30°C by heated ventilation and copper wool insulation and recorded via a thermocouple inserted in the box. Thermocouples were attached to digital temperature indicators with 12-bit (box temperature) or 16-bit (skin and rectal temperature) digital to analog converters (Omega Engineering Inc.) that provided a linear voltage proportional to temperature. Data were collected on a VAXstation 4000 VLC (Digital Equipment Corporation; DEC, Maynard, MA) using DEC Realtime Test Integrator software and a multiplexed 16-bit analog-to-digital converter (ADC488/16; IOTEC, Cleveland, OH). After initial collection, data were analyzed using RS/1 (BBN Software, Cambridge, MA).

During sessions in which airway conductance (SGaw) was measured, a latex dam (1.5 mm thick dental dam) was fit snugly around the monkey's neck and lay flush with the neck plate; when compressed by a helmet it assured an air-tight connection. Filtered air or aerosol test atmospheres were delivered under positive pressure to the helmet at a rate of 5 l/min via a three-way ball valve and tubing assembly (2100 Series, Hans Rudolph Inc., Kansas City, MO). The atmosphere exited through a separate port on the helmet into the plethysmographic box and then through a valve into an exhaust system. The method described by Agrawal (1) was modified to measure SGaw in monkeys: inhaled volume and flow were measured after temporarily closing all valves immediately following exposure to air or to aerosols. Respiratory frequency and airflow were measured using a pneumotachograph (model 0, A. Fleisch, Switzerland), which was attached to a differential pressure transducer (MP45-14-87/ ± 2 ml H_2O, Validyne Engineering Co., Northridge, CA). Box pressure was also measured using a similar differential pressure transducer. The signals of airflow and box pressure were digitized by an analog/digital converter (MacAdios; GW Instruments, Somerville, MA) collected with Superscope II software (GW Instruments) using a Macintosh computer system (Apple Computer, Cupertino, CA).

Data Analysis

Pulmonary function change was determined using the method of Agrawal (1). Changes in plethysmograph box pressure reflected mean alveolar pressure changes due to expansion and compression of intrathoracic gas. Specific airway conductance (SGaw) was calculated by measuring the changes of the airflow (Δflow) and box pressure signals (Δvolume) during the transition phase between inspiration and expiration. During this phase, the change in lung volume is minimal and temperature and humidity artifacts are negligible. SGaw was calculated as follows:

$$\text{SGaw} = \frac{\Delta\text{flow}}{\Delta\text{volume}} \times \frac{1}{P_B - P_{H_2O}}$$

where P_B is the barometric pressure and P_{H_2O} is the water vapor pressure at body temperature.

Untransformed data for heart rate, rectal, and skin temperatures are reported as the median for 0.5- or 5-s collection periods.

RESULTS

Operating Characteristics of the Evaporation-Condensation Generator

Increasing the temperature of the boiler increases the vapor pressure of cocaine (Fig. 2, top), ultimately increasing particle size (Fig. 2, bottom). Increasing temperature also increases

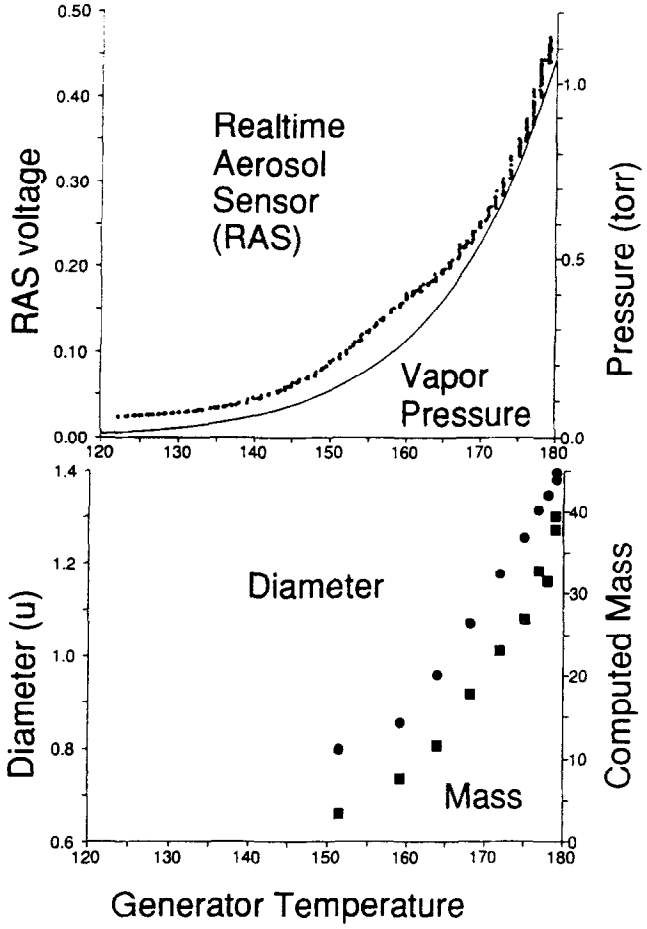


FIG. 2. Effects of temperature on cocaine airborne mass and particle diameter. Top: voltage produced from realtime aerosol sensor as a function of temperature (°C) of boiler. The vapor pressure function for cocaine base is plotted on the same axes ($\log P_{TORR} = (-5884/T_{Kelvin}) + 13.02$; Lawrence et al., 1984) (10). Temperature and voltage were measured at a fixed rate; the "stepped" appearance is a result of one instrument's limited resolution in this temperature range. Bottom: mass median optical diameter (●) and computed mass (■) (arbitrary units) derived from the optical particle analyzer as a function of boiler temperature.

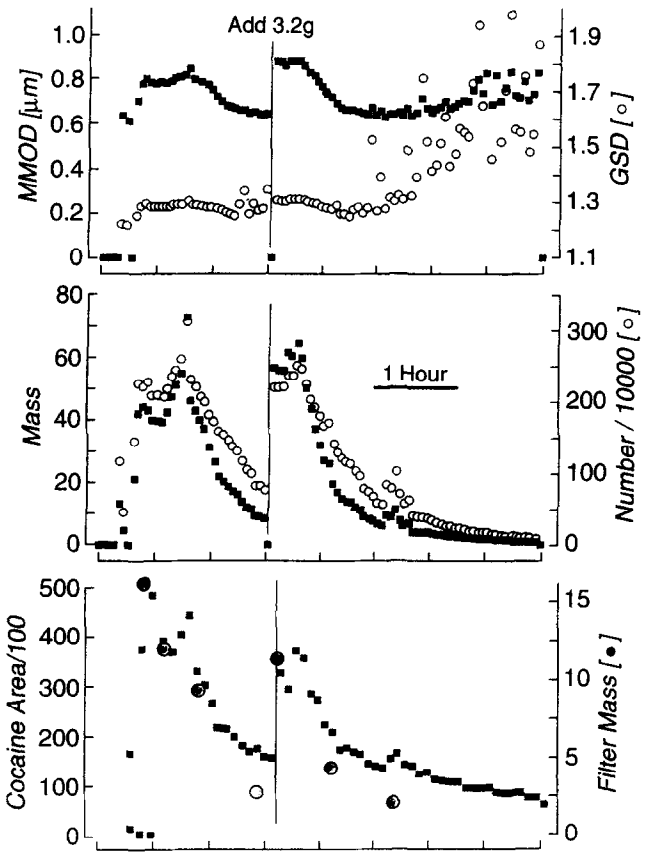


FIG. 3. Operating the generator with the addition of fixed amounts of cocaine permits the estimation of cocaine disappearance rates. Note that particle diameter becomes smaller with reduction of amount of cocaine in the boiler. Top panel: mass median optical diameter (MMOD) (left axis, ■); geometric standard deviation (GSD; right axis; ○). Middle panel: optical particle sizing estimate of airborne mass (left axis, ■), or particle number (right axis, ○). Bottom panel: airborne mass (peak area) estimated by air sampling gas chromatograph (left axis, ■), or from filter mass (mg/l of air, right axis, ○).

airborne concentration (Fig. 2, top), and the rate of loss from the reservoir. As the depth of liquid in the reservoir gets shallower, the carrier gas does not achieve saturation, and the vapor concentration falls; adding cocaine increases both particle size and mass (Fig. 3). As the number of condensation nuclei increases, the available vapor is distributed across more particles, so they may be smaller. If too few nuclei are present, self-nucleated large particles occur, but most of the vapor may condense on the walls of the condensation zone (wall loss). If significant wall loss begins, dendritic crystal growth occurs concentrically along tubing surfaces, concentration falls progressively, and particles tend to be smaller. The distribution may be more disperse if rocks break loose from the dendritic accumulations. Prevention of wall loss is a significant consideration in the design of systems intended for stable operation.

Adding more cocaine to the boiler is associated with increasing particle diameter, number, and mass. To determine the rate of cocaine loss from the reservoir during generation

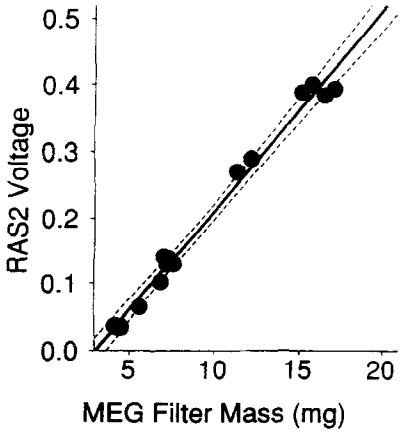


FIG. 4. Generation of a pure methylecgonidine test atmosphere using the boiler. The nephelometer voltage (RAS2) was linearly related to the amount of methylecgonidine collected on the filter and in line charcoal tube following a 1 liter sample of the test atmosphere.

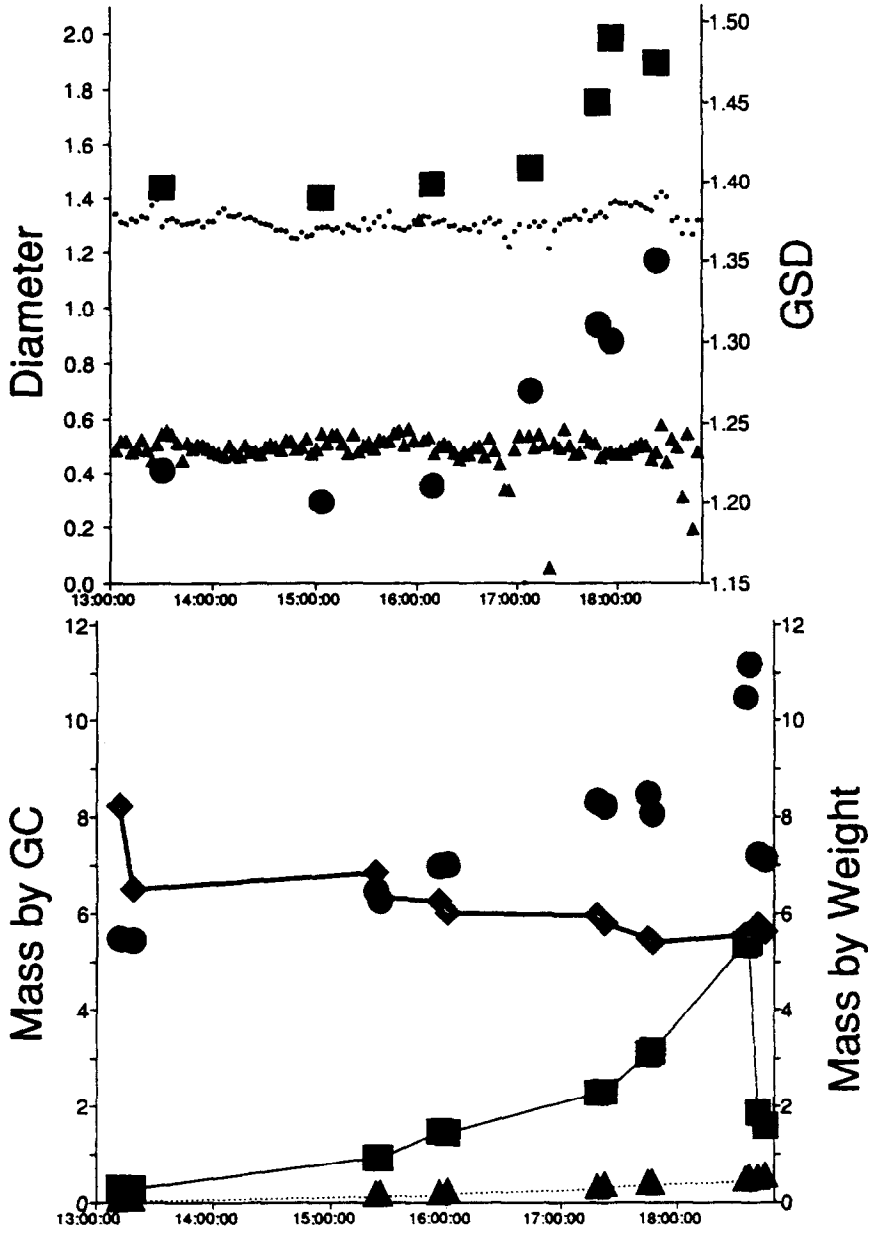


FIG. 5. Record of particle generation across time when MEG was coated onto the cocaine particle by increasing the temperature of the coating vessel across the session. The atmosphere was monitored by the optical particle sizing instrument, by air sampling to determine the chemical composition and amount of each chemical present, and to characterize median aerodynamic diameter and dispersion of the particles with the Mercer cascade impactor. Top panel: the coating technique successfully resulted in particles with greater mass median aerodynamic diameter (■) comprised of cocaine and MEG measured using the impactor at the point of delivery to the experimental animal. Because MEG has a much higher vapor pressure than cocaine at room temperature, it evaporated from the cocaine particle as the atmosphere was diluted sufficiently to permit accurate optical characterization of the particle; hence, the cocaine optical particle diameter (·) remained constant at 1.35 μm across the 6 h of operation of the instrument, while the geometric standard deviation of the particle size distribution remained constant at 1.23 (▲). The geometric standard deviation increased somewhat with increasing diameter, as measured with the impactor (●), although it was still monodisperse. Bottom panel: the airborne mass was determined by drawing one liter of the test atmosphere through the filter, weighing it (●), and then analyzing its components: ◆ Cocaine, ■ MEG, ▲ benzoic acid. Total mass increased with increasing coater temperature, reflecting increased MEG content while cocaine levels remained constant. The temperatures at the time of Mercer and filter samples were: 30.19, 65.55, 77.10, 89.85, 101.60, 105.24, and 116.45°C.

of maximal levels of cocaine (about 15 mg/l of air) an experiment (Fig. 3) was done with a constant number of condensation nuclei, a large initial load of cocaine, a shallow bead bed, and boiler temperature at 165°C. When the concentration had fallen approximately 70%, we added another 3.2 g of cocaine, producing an increase in concentration as measured by all instruments, and a 0.2 μm increase in particle diameter (Fig. 3, top panel). When these mass and number disappearance functions (Fig. 3, middle panel) were examined on semilog coordinates, the disappearance slopes for the two loads were all parallel and offset by 80 min, giving a mass consumption rate of 2.4 g/h. Furthermore, all the different indices were parallel with each other, except for the chromatographic function whose slope was slightly lower and associated with a long sampling line. To maintain high levels (<10 mg/l) of cocaine generation for hours thus requires topping up the boiler at 2.4 g/h; we used the heated syringe and syringe pump for this purpose. In the absence of continuous addition, concentrations above 10 mg/l could only be sustained for approximately 20 min (Fig. 3, bottom).

Generation of MEG Aerosols

MEG aerosols can be generated using the same techniques as those used for the generation of cocaine; the liquid methylecgonidine can be generated as the principal aerosol, rather than as a coating on a cocaine aerosol. On one representative day, the boiler temperature was set to 107.2°C, 15 ml of MEG were infused into the hot finger, and the infusion rate was maintained at 4.66 ml/h. Three concentrations were established by dilution: 16.12 ± 0.43 (mean ± SE); 7.3 ± 0.12; and 4.33 ± 0.085 mg/l. Further dilution was necessary to reduce the particle number concentration to the operating range of the optical particle analyzer. Unfortunately, the particles evaporated under these conditions. Thus, we turned to the Mercer impactor to characterize these aerosols, and the corresponding particle MMAD (σ_g) were: 1.5 μm (2.18), 1.25 (2.23), and 1.62 (2.52). A linear relationship was achieved between the airborne concentration measured with a filter in series with a charcoal tube (22) (1 min at 1 liter/min), and the nephelometer (RAS2) voltage (Fig. 4). This relationship permits the rapid adjustment of concentration using optical techniques following calibration with more laborious chromatographic determinations.

Coating of Particles With, and Evaporation of, MEG From the Cocaine Droplet Within the Analytical System

This condensation aerosol generator can be used to coat the pyrolyzate MEG onto cocaine base droplets by placing MEG in the three-necked coating flask, and increasing its temperature. During a session in which temperature was increased in steps, measurement of the atmosphere with filters and charcoal tubes (22) (filled circles bottom panel, Fig. 5) and inertial impactors (filled squares, top panel Fig. 5) indicated that mass and particle diameter increased with increasing coater temperature. The nephelometer measured the airborne mass after one approximately 10-fold dilution that was sufficient to keep the maximum concentration in the linear zone of operation of the instrument; the instrument showed increasing mass with increasing temperature (data not shown). However, the optical particle sizing instrument required approximately a 1000-fold dilution of the atmosphere, thus increasing the evaporation of the pyrolyzate from the particle surface. Note the extreme stability of the instrument's count of the number

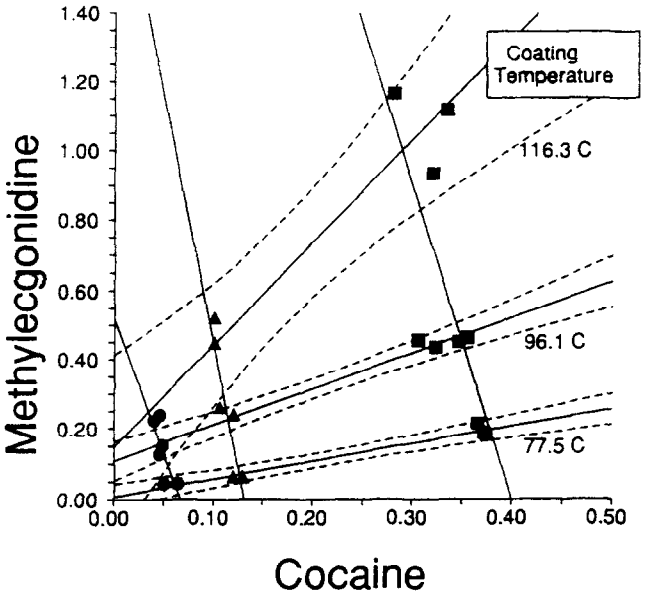


FIG. 6. Simultaneous control of relative concentration of cocaine and methylecgonidine by varying cocaine dilution (■ × 1, ▲ × 0.294, ● × 0.132) and methylecgonidine coating temperature (77.5, 96.1, and 116.3°C). Units are peak area. Temperature was increased stepwise across the session, and cocaine dilution was varied in discrete steps within a coater temperature setting. The decrease in cocaine concentration with increasing temperature of the coater was because of an uncorrected downward trend in cocaine concentration during this experiment. Regression lines were fit to the data collected at each temperature, and are bounded by their respective 95% confidence limits.

of particles as well as its estimation of particle diameter, in this case, the remaining cocaine particle.

Independent control of cocaine and MEG concentrations were demonstrated during a session in which three coating temperatures were examined, and the cocaine atmosphere was diluted in the same (i.e., ABCA) order within each temperature condition. Particle size measured optically was constant, reflecting evaporation of MEG from the cocaine particle within the analytical instrumentation (not shown). However, particle counts, computed masses, and the filter analyses tracked these manipulations. Figure 6 presents the MEG peak area determined by chromatographic analysis of the filter samples against cocaine peak area determined for the same samples. Explicit control of the ratio of the two agents was achieved by simultaneous manipulation of the extent of cocaine dilution and coater temperature.

Individual two-way analyses of variance were undertaken on six dependent measures obtained in this study of coating and dilution, and the analysis of variance tables are presented in Table 1. The mass of cocaine determined by gas chromatography was controlled by the dilution system; this parameter accounted for 97% of the variance in cocaine levels. Because the MEG coating temperature was confounded with time, the change in airborne concentration of cocaine is most likely a change in the output of the generator per se over the course of the experiment, and was not an effect of the coating operation. The F statistics for dilution and MEG temperature are based on an additive model and use the residual component for the denominator of the F-ratio. There was only marginal evidence that dilution affected diameter ($p = 0.056$), but there

TABLE 1

ANALYSIS OF VARIANCE TABLES FOR INDIVIDUAL DEPENDENT MEASURES DURING THE EXPERIMENT IN WHICH THE RATIO AND CONCENTRATION OF METHYLECGONIDINE AND COCAINE WERE VARIED BY CONTROLLING COATER TEMPERATURE AND DILUTION

Source	DF	%V	F	p	DF	%V	F	p
		Filter Mass				Particle Count		
Dilution	2	86.38	384.73	‡	2	58.85	14.25	‡
Temperature	2	9.25	41.21	‡	2	1.90	0.46	
Residual	19	4.37			19	39.25		
Interaction	4	2.68	5.97	†	4	7.92	0.95	
Error	15	1.68			15	31.33		
Total	23	100.			23	100.		
		Cocaine				Methylecgonidine		
Dilution	2	96.92	478.47	‡	2	44.33	282.73	‡
Temperature	2	1.16	5.71	*	2	37.24	237.49	‡
Residual	19	1.92			19	18.43		
Interaction	4	0.33	0.77		4	17.25	55.02	‡
Error	15	1.60			15	1.18		
Total	23	100.			23	100.		
		Particle Diameter				Geometric Standard Deviation		
Dilution	2	15.14	3.35		2	7.46	0.97	
Temperature	2	41.98	9.30	†	2	19.44	2.53	
Residual	19	42.90			19	73.10		
Interaction	4	0.	0		4	0	0	
Error	15	42.90			15	73.10		
Total	23	100.			23	100.		

%V is the percent of variance accounted for by each parameter.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

was strong evidence that MEG temperature did ($p = 0.0015$). Dilution and MEG coater temperature affected airborne mass determined gravimetrically; the interaction was also significant. The same finding held true for MEG concentration determined chromatographically. Particle count was affected by dilution, but not by coater temperature, and there was no interaction. Dilution and coating temperature is without effect on dispersion of particle size distribution, and no interaction was observed.

Acute Effects of Inhaled MEG or Cocaine in Squirrel Monkeys

The effects of exposure to the condensation aerosols of MEG free base on SGaw, heart rate, and rectal temperatures are presented in Fig. 7. A decrease in SGaw was apparent in all monkeys breathing atmospheres containing a condensation aerosol of MEG free base (Fig. 7, bottom panels). This decrease was observed within 5-min of exposure; thereafter, SGaw returned to preexposure levels within 25 min (Fig. 7, bottom panels). Within seconds of exposure to MEG free base, a decrease in heart rate was observed in two monkeys (61 and 1034; Fig. 7, top left and right panels), and an increase in heart rate in one monkey (63; Fig. 7, top center panel). Decreases in rectal temperature were also observed following exposure to MEG at all concentrations in monkeys 63 and 1034, while only the highest concentration decreased rectal temperature in monkey 61 (Fig. 7, middle panels).

The effects of exposure to cocaine condensation aerosols are illustrated in Fig. 8 for monkey 1034. Cocaine induced an increase in heart rate (Fig. 8, top panel) and rectal temperature (Fig. 2, middle panel) while decreasing SGaw (Fig. 8, bottom panel). This monkey was reexposed to cocaine during the same session. A small increase in rectal temperature and a decrease in heart rate was observed in the 2–3 min after the second exposure to cocaine; this was followed by a delayed increase in heart rate. A reduction in SGaw was still apparent after the second exposure to cocaine.

DISCUSSION

Cocaine condensation aerosols can be generated by feeding a carrier gas through a condensation nucleus generator, which provides a supply of seeds for the subsequent formation of a drug cloud when the vapor condenses upon them. This seeded carrier gas is forced through a reservoir of molten cocaine and the resultant mixture is then passed into a second heated zone to insure that all of the drug is in the vapor state. The vapor and condensation nuclei then pass into a downward flowing chimney in which the material cools and condenses to form the aerosol test atmosphere. If the vapor concentration is constant, providing a greater number of seeds would result in smaller particles. If the cocaine pyrolysis product, MEG, is desired at elevated levels in the test atmosphere, it can be coadministered by passing the cocaine aerosol through a coating zone maintained at a lower temperature. Temperature controls the vapor pressure of the pyrolyzate, which will condense on all surfaces, for instance, the cocaine particle surface and the condenser wall. The total cocaine particle surface determines the amount of pyrolyzate that will be carried out of the coater. In this manner, a desired cocaine/MEG mixture can be produced continuously on a given day.

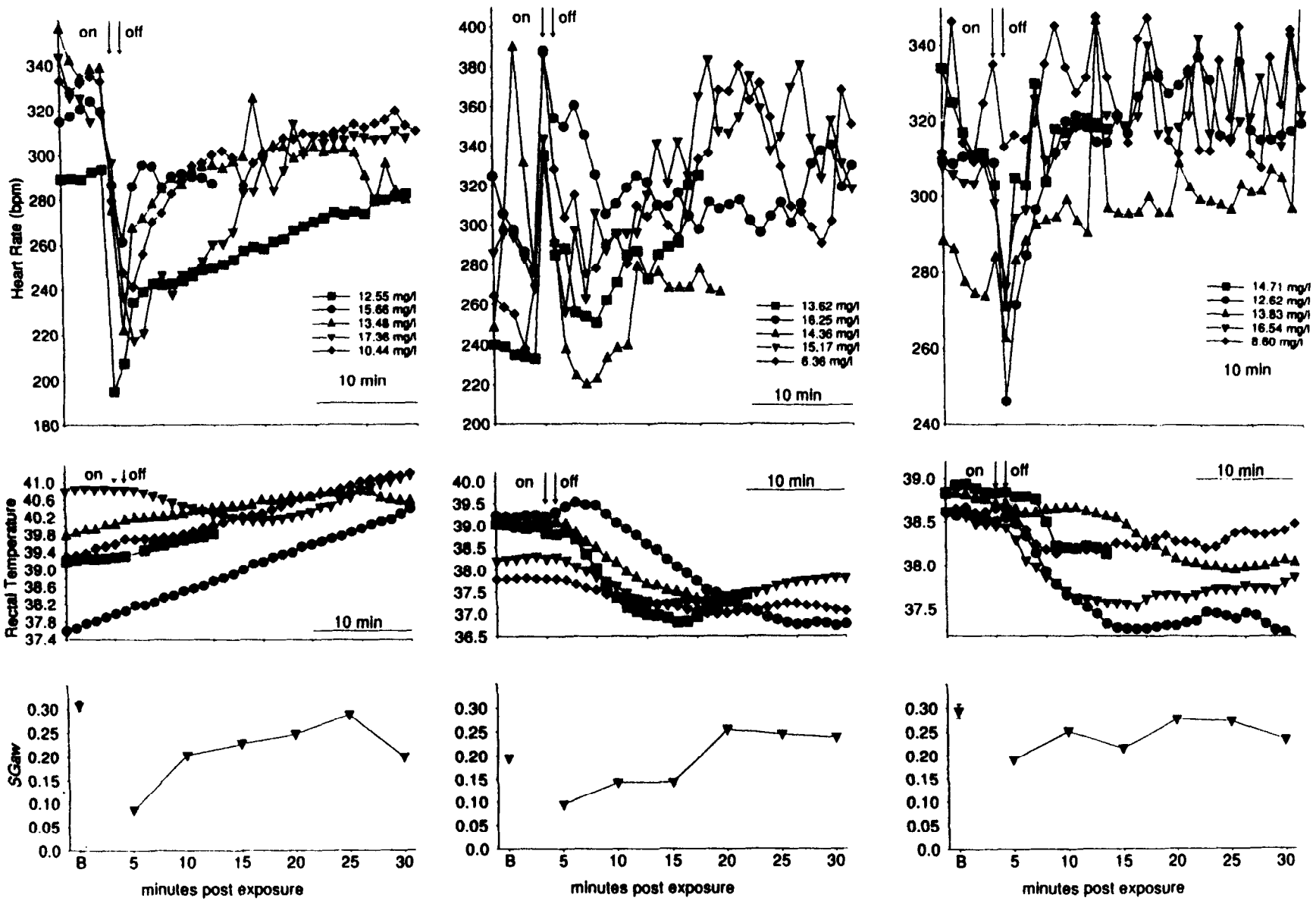


FIG. 7. Effects of inhalation of a condensation aerosol of MEG free base in squirrel monkeys 61 (left panels), 63 (center panels), and 1034 (right panels). The top panels show effects on heart rate (beats per minute); middle panels, rectal temperature (°C); bottom panels, specific airway conductance (SGaw; s^{-1} cmH_2O^{-1}). In the top and middle panels, arrows indicate the onset (on) and offset (off) of exposure to the condensation aerosols of MEG; each exposure lasted 1 min. Note that the time course shown in the top and middle panels is different from that shown in the bottom panel, which shows the mean (± SEM) of four to five baseline SGaw measurements (points above B) taken in animals prior to exposure to MEG and at 5–30 min after MEG exposure; only one determination of SGaw was made in each animal at each time point following MEG exposure.

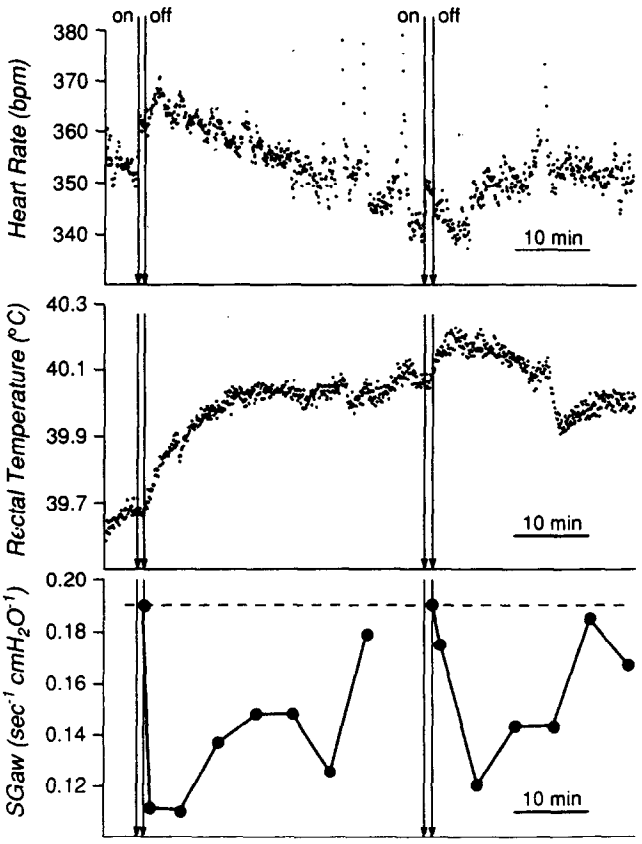


FIG. 8. Effects of inhalation of condensation aerosols of cocaine in squirrel monkey 1034. The top panel show effects on heart rate (beats per minute); middle panel, rectal temperature (°C); bottom panel, specific airway conductance (SGaw; s^{-1} cmH_20^{-1}). Arrows indicate onset (on) and offset (off) of two 1-min exposures to 5.18 mg/l of cocaine free base aerosol.

Stamatakis et al. (17) identified a number of research needs in aerosol processing, and the present effort has provided a strategy for addressing the problem of continuous process monitoring at the high airborne mass concentrations. Most particle sizing and counting instruments are designed for this purpose. By incorporating several stages of dilution, we have been able to adjust the airborne concentration of materials to within the operating range of several instruments, and, thus, have been able to provide estimates of airborne concentration as well as of particle size and homogeneity.

Aerosols are intrinsically unstable in time (16), and dilution was demonstrated in the present article to be associated with the removal of the more volatile pyrolyzate, MEG, from the particulate phase before the atmosphere was sufficiently reduced in number concentration to permit optical estimation of diameter without significant coincidence error, that is, the simultaneous presence of more than a single particle in the measurement volume of the instrument. The evaporation of MEG from the cocaine particle in this analytical system systematically replicates the phenomena observed in model crack pipes (22); we found that MEG occurred in crack smoke at concentrations up to 5.0%, that the cocaine and MEG existed together as one particle, and that MEG in the vapor phase persisted in a chamber after the disappearance of cocaine, consistent with its evaporation from surfaces. With the use of multiple instruments and crosscalibration exercises, it was possible to achieve with a high degree of repeatability and certainty aerosol test atmospheres of defined chemical composition, proportion of vapor present, median particle diameter, and degree of dispersion of the particle size distribution.

Inhaled MEG displays effects on cardiovascular and pulmonary function in nonhuman primates. Notably, inhalation of the condensation aerosol of MEG free base induces bronchoconstriction and displays effects on cardiovascular function. Inhalation of MEG base produced effects on airway conductance that were like those of cocaine base. We have also observed that inhalation of MEG base induces bronchoconstriction in guinea pigs but the nebulized fumarate salt of MEG induces bronchoconstriction in guinea pigs only at concentrations that are near saturation, are acidic, and have a relatively large particle size (4). The physical size of the condensates or the alkaline challenge posed by the free base may present direct irritation to the airways. Within seconds of inhalation exposure to MEG, changes in heart rate and temperature were also observed and were somewhat concentration related.

Inhalation of cocaine free base also induced a decrease in SGaw, and changes in heart rate and temperature. MEG is produced when cocaine base is heated under the conditions described by Hatsukami et al. [(6,22); Hatsukami, personal communication]. MEG is also produced when the condensation aerosol of cocaine is generated (21,22). Therefore, the relative contributions of MEG and cocaine to the observed effects of inhalation of the condensation aerosol of cocaine in the present study, or of cocaine base smoke in other studies (6), remains to be determined [(22), Hatsukami, personal communication].

While the amount of MEG produced during pyrolysis of crack may represent 2% or less of the total inhaled mass [(22); Hatsukami, personal communication], given the present results and the fact that significant amounts of MEG have been found in the urine of subjects smoking crack (8,9), a contribution of MEG cannot be excluded when considering the physiological or psychological sequelae of smoking crack cocaine. Only preliminary reports of the behavioral effects of MEG exist (12,20) and they suggest that MEG is not cocaine-like. MEG's physiological effects after smoking crack are likely to reflect a complex interaction between reflexly induced events as well as direct effects on as yet undetermined cellular components. The contribution of MEG to persistent changes in pulmonary function implicated by studies of humans with self-reported histories of crack abuse is unknown. However, the demonstrated activity of MEG suggests that it may play a role in acute toxicity reactions and possibly persistent lung injury.

In summary, we describe here the development of technologies that enable generation of multicomponent condensation aerosol test atmospheres of known chemical composition, defined particle size, and of very narrow dispersion suitable for studies with laboratory animals. The generation of stable airborne concentrations for administration upon demand across an experimental day enables the study of acute tolerance phenomena, as well as the evaluation of the joint effects of cocaine and MEG. MEG has been demonstrated to be a noncompetitive antagonist of acetylcholine and other spasmogenics in vitro (5), and to have pronounced cardiopulmonary effects in laboratory animals [(4), this report]. The present technology permits the study of interactions of cocaine and MEG by the inhalation route, thus enabling the separation of route-dependent effects, if any, from those attributable to pharmaco-

logical affects observed following distribution in the systemic circulation.

ACKNOWLEDGEMENTS

This work was supported by Grants DA00623, DA04438, and KO2-DA00117 from the National Institute on Drug Abuse. R. W. Wood was the principal investigator. The authors wish to thank Drs. C. Schindler and B. Cohen for extensive consultation. Ms. Dawn Piccola and Mr. Min Hua Zhong are thanked for technical assistance. All animals used in this study were maintained in accordance with the guidelines of the Animal Care and Use Committee, New York University and of the "Guide for Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication No. (NIH)85-23, revised 1985.

REFERENCES

1. Agrawal, K. P. Specific airways conductance in guinea pigs Normal values and histamine induced fall. Respir. Physiol. 43:23–30; 1981.
2. Asgharian B.; Wood, R.; Schlesinger, R. Empirical modeling of particle deposition in the alveolar region of the lungs: A basis for interspecies extrapolation. Fundam. Appl. Toxicol. 27:232–238; 1995.
3. Boni, J. P.; Barr, W. H.; Martin, B. R. Cocaine inhalation in the rat: Pharmacokinetics and cardiovascular response. J. Pharmacol. Exp. Ther. 257:307–315; 1991.
4. Chen, L. C.; Graefe, J. F.; Shojaie, J.; Willetts, J.; Wood, R. W. Pulmonary effects of the cocaine pyrolysis product, methylecgonidine, in guinea pigs. Life Sci. 56:PL7–PL12; 1995.
5. El-Fawal, H. A. N.; Wood, R. W. Airway smooth muscle relaxant effects of the cocaine pyrolysis product, methylecgonidine. J. Pharmacol. Exp. Ther. 272:991–996; 1995.
6. Hatsukami, D.; Keenan, R.; Carroll, M.; Colon, E.; Geiske, D.; Wilson, B.; Huber, M. A method for delivery of precise doses of smoked cocaine-base to humans. Pharmacol. Biochem. Behav. 36:1–7; 1990.
7. Hinds, W. C. Aerosol technology: Properties, behavior, and measurement of airborne particles. New York: J. Wiley; 1982.
8. Jacob, P., III; Lewis, E. R.; Elias-Baker, B. A.; Jones, R. T. A pyrolysis product, anhydroecgonine methyl ester (methylecgonidine), is in the urine of cocaine smokers. J. Anal. Toxicol. 14:353–357; 1990.
9. Jacob, P., III; Jones, R. T.; Benowitz, N. L.; Shulgin, A. T.; Lewis, E. R.; Elias-Baker, B. A. Cocaine smokers excrete a pyrolysis product, anhydroecgonine methyl ester. J. Toxicol. Clin. Toxicol. 28:121–125; 1990.
10. Lawrence, A. H.; Elias, L.; Authier-Martin, M. Determination of amphetamine, cocaine, and heroin vapor pressures using a dynamic gas blending system and gas chromatographic analysis. Can. J. Chem. 62:1886–1888; 1984.
11. Muir, D. C. F. The production of monodisperse aerosols by a LaMer-Sinclair generator. Ann. Occup. Hyg. 8:233–238; 1965.
12. Newman, A. H.; Allen, A. C.; Witkin, J. M.; Izenwasser, S.; Mash, D.; Katz, J. L. The thermal decomposition product of crack, AEME, and analogs do not appear to contribute acutely to the pharmacological or toxicological actions of cocaine. Med. Chem. Res. 4:93–110; 1994.
13. Prodi, V. A. A condensation aerosol generator for solid monodisperse particles. In: Mercer, T.; Morrow, P. E.; Stober, W., eds. Assessment of airborne particles. Springield, IL: Charles C. Thomas; 1972:169–181.
14. Schlesinger, R. B. Comparative deposition of inhaled aerosols in experimental animals and humans: A review. J. Toxicol. Environ. Health 15:197–214; 1985.
15. Sinclair, D.; LaMer, V. K. Light scattering as a measure of particle size in aerosols. Chem. Rev. 44:245–267; 1949.
16. Soderholm, S. Unstable aerosols. In: Advances in air sampling. Chelsea, MI: American Conference of Governmental Industrial Hygienists, Lewis Publishers; 1988:161–174.
17. Stamatakis, P.; Natalie, C. A.; Palmer, B. R.; Yuill, W. A. Research needs in aerosol processing. Aerosol Sci. Technol. 14:316–321; 1991.
18. Swift, D. L. A study of the size and monodispersity of aerosols produced in a Sinclair-LaMer generator. Ann. Occup. Hyg. 10:337–348; 1967.
19. Willeke, K.; Baron, P. A. Aerosol measurement: Principles, techniques, and applications. New York: Van Nostrand Reinhold; 1993.
20. Willetts, J.; Chen, L. C.; Graefe, J. F.; Wood, R. W. Effects of methylecgonidine on acetylcholine-induce bronchoconstriction and indicators of lung injury in guinea pigs. Life Sci. 57:PL225–PL230; 1995.
21. Wood, R. W.; Graefe, J. F.; Fang, C. P.; Shojaie J. Liquid methylecgonidine coats the crack droplet. In: Harris, L. S., ed. Problems of drug dependence, 1993: Proceedings of the 55th annual scientific meeting, the college on problems of drug dependence, Inc., Vol. II, NIDA Research Monograph 141. Rockville, MD: DHSS NIH Publication No. 94-3749; 1994:321.
22. Wood, R. W.; Shojaie, J.; Fang, C. P.; Graefe, J. F. Methylecgonidine coats the crack particle. Pharmacol. Biochem. Behav. 53:57–66; 1996.