A noninvasive method for delivering controlled doses of nicotine via cigarette smoke

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A cigarette-smoke delivery system is described in which nicotine dosage was delimited by having the subject inhale a measured amount of smoke to a predetermined depth and duration of inhalation. A plastic syringe was used to "inject" a specified amount of cigarette smoke into the subject's mouth, and an airbag containing 1 liter of air was used to provide a "chaser" with a fixed volume of inhalation for the smoke. Using plasma nicotine boost as an indicator, dose control was found to be nearly linear for the three dose levels employed; in the high-dose condition, plasma nicotine levels were moderately consistent within subjects over three successive administrations. Between-subject variability was considerably greater than within-subject variability, however, suggesting that each smoker obtained a characteristic nicotine boost that reflected individual differences in nicotine pharmacokinetics in addition to nicotine intake per se.

It has been recognized for some time in pharmacological research that scientific understanding of drug action generally starts with the ability to administer known, controlled doses of the substance of interest, and with the accurate determination of levels in biological samples. Many of the gains in knowledge about cigarette smoking and nicotine self-administration over the past decade can be attributed to increasing sophistication in the quantitation of levels of nicotine and its chief metabolite, cotinine, in biological fluids (U.S. Department of Health and Human Services, 1988). Unfortunately, progress in the development and dissemination of techniques for controlled nicotine dosing, particularly in the case in which nicotine is inhaled via cigarette smoke or nicotine aerosol, has not kept pace with improvements in reliable and sensitive assays for nicotine.

At last three criteria must be met for a nicotine dosing method to be regarded as a satisfactory tool for understanding the effects of nicotine (C. S. Pomerleau, Majchrzak, & O. F. Pomerleau, 1989b): (1) The method must be safe and easy to use; (2) specified doses must be reliably and reproducibly delivered as verified by plasma nicotine assays, regardless of factors such as exposure to

stress or degree of nicotine deprivation or satiation that might affect nicotine intake; and (3) ideally, the pharmacodynamics of administration should resemble the spike-and-decay pattern of cigarette smoking, since many of its characteristic reinforcing effects seem to be based on this pattern (O. F. Pomerleau & C. S. Pomerleau, 1984). In addition to the direct administration of nicotine intravenously (Benowitz, Jacob, Jones, & Rosenberg, 1982; Feyerabend, Ings, & Russell, 1985), various strategies have been used to accomplish these objectives, ranging from simple methods such as changing the nicotine content of cigarettes (Benowitz, Kuyt, & Jacob, 1982) and fixing puff duration and puff interval via instructional control (C. S. Pomerleau, Majchrzak, & O. F. Pomerleau, 1989b) to more highly elaborate methods. An example of the latter is a technique developed by Zacny, Stitzer, Brown, Yingling, & Griffiths (1987), involving a microcomputer, smoking topography sensors (for puff volume, number, and spacing), a strain gauge on the chest to determine depth of inhalation, and auditory feedback to the smoker to modify puff and inhalation parameters (Zacny et al., 1987).

In the present report, we describe a cigarette-smoke delivery system in which the experimenter delimits nicotine dosage by having the subject inhale a fixed amount of smoke to a predetermined depth and duration of inhalation. The approach was developed as a compromise between simple methods of dose control, which have generally been shown to be inadequate, and more technologically demanding methods, which may not be practical in many circumstances.

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METHOD

Subjects

The subjects were 9 male heavy smokers, in good health and not on medications, who were recruited from the local community through newspaper advertisements. They were required to be smoking at least 15 cigarettes per day and to have smoked for at least 5 years (menthol-cigarette smokers were excluded). Mean ($\pm SD$) age was 31.0 ± 7.9 years, and mean duration of smoking was 15.0 ± 6.9 years. The subjects smoked a mean of 27.8 ± 11.2 cigarettes per day, had mean scores of 7.2 ± 2.4 on the Fagerstrom Tolerance Questionnaire (an 11-item scale intended to measure nicotine dependence; see Fagerstrom, 1978; C. S. Pomerleau, Majchrzak, & O. F. Pomerleau, 1989a), and had mean plasma levels of 357.0 ± 175.1 ng/ml of cotinine, characterizing them as heavy smokers (C. S. Pomerleau, O. F. Pomerleau, Majchrzak, Kloska, & Malakuti, in press). The subjects ranged in weight from 56 to 103 kg, with a mean weight of 79 ± 16 kg. They were paid \$75 on completion of the entire experimental sequence.

Apparatus

Figure 1 shows the apparatus, which is basically a smoke-transfer device constructed from plastic connectors (inner diameter, 5 mm) and flexible rubber tubing (Tygon; inner diameter, 9 mm). Smoke was contained in the syringe as briefly as possible, and tubing was kept short to minimize smoke/nicotine adsorption and dead space volume. The airbag (22-mm breathing bag; Vacu-metrics, Inc., Ventura, CA) was inflated with compressed

air to a 1-liter volume (determined using a calibration band around the circumference).

The cigarettes used for dosing were either a high-nicotine (2.5-mg) cigarette (research cigarette 2R1; Tobacco and Health Research Institute, University of Kentucky) or a zero-nicotine cigarette fabricated from cocoa husks ("Free," International Brands, Inc., Los Gatos, CA). One or 2 days prior to use, the cigarettes were placed in a humidor (the bottom of which contained a supersaturated solution of ammonium nitrate) at room temperature.

Procedure

The dosing procedure was as follows: The 60-cc plastic syringe was used to extract (at a constant rate, over 5 sec) a predetermined amount of smoke from a freshly lit high-nicotine or zero-nicotine cigarette. A short length of flexible rubber tubing connected to the syringe was used to withdraw smoke from the cigarette (using a removable plastic connector as cigarette holder). To administer the smoke, the syringe tubing was coupled to a plastic connector secured to a 21-cm length of tubing attached to a freshly inflated 1-liter airbag. The airbag/tubing was handed to the subject, who used the thumb and forefinger to pinch shut a length of tubing proximal to the airbag (thereby preventing premature release of air) and placed the opposite end of the tubing in his mouth. The procedure consisted of the experimenter's "injecting" a predetermined amount of smoke into the subject's mouth using the syringe; this defined a "puff." The "puff" was held in the mouth during the 2-sec injection; the smoke was rapidly inhaled at the same time as the subject released the flexible tubing (to allow the liter of air to serve as a "chaser" for the smoke, thereby diluting the smoke within a fixed volume of inhalation). After 3 sec, the subject was told to exhale. A complete administration consisted of 10 such doses, at 1-min intervals. The high dose consisted of 60-cc "puffs" using smoke from the high-nicotine cigarette; the medium dose, 30-cc "puffs" from the high-nicotine cigarette plus 30 cc air; the zero dose, 60-cc "puffs" from the nicotine-free cigarette.

The subjects were trained to smoke using the airbag procedure in a screening session. The formal study consisted of five .5-h sessions (one zero-nicotine, one medium-nicotine, and three high-nicotine sessions) separated by at least 1 day. Half an hour before the start of a session, the subjects were asked to smoke a usual cigarette in order to ensure a standard level of minimal deprivation. Immediately after smoking, a line was inserted for blood withdrawal, electrodes for monitoring heart rate were emplaced, and a temperature sensor was taped to the left index finger. The subject inhaled smoke using the airbag procedure for the first 10 min of each session and then rested quietly for the remaining 20 min. Before and after smoking, desire to smoke and smoking withdrawal symptoms (overall) were queried via a visual analogue scale (0 to 100 in arbitrary units) presented on an Amiga computer; the subject used a mouse to specify intensity. A Latin square design was used to determine

Figure 1. Airbag apparatus.

the order of the first three conditions (zero, medium, and first high-nicotine dosing); two additional high-nicotine dosing sessions completed the sequence. The subjects were blind to dose condition.

Data analysis. The study was conceived as two separate subexperiments: (1) To assess parametric dose control, the zero-, medium-, and first high-nicotine sessions were analyzed. To determine whether nicotine boosts for these three sessions were significantly different from one another, a one-way repeated measures ANOVA was conducted using change scores (plasma nicotine sample at Minute 10 minus Minute 0). A similar analysis was conducted on physiological and subjective data taken at the same time, in order to verify similarity to the effects of cigarette smoking. Coefficients of variation ($CV = SD/mean \times 100$) were calculated for nicotine change scores for each of the 3 days to evaluate between-subject variability in nicotine intake; the coefficient of variation is a unit-free measure of variability scaled to correct for different magnitudes of response. (2) To assess ability to reproduce a given dose, change scores for the three high-nicotine sessions were analyzed using one-way repeated measures ANOVAs, and CVs were calculated as described for Subexperiment 1. In addition, in order to examine the extent to which subjects dosed consistently, Spearman correlation coefficients and within-subject CVs were calculated using each subject's nicotine change scores across the 3 high-nicotine days.

Nicotine sampling. Blood samples were withdrawn unobtrusively from the right median antecubital vein at 0, 10, and 15 min, using an indwelling catheter leading to heparinized 1-m infusion-exfusion tubing, and collected in 10-ml EDTA monovettes. Following centrifugation at 2500 rpm for 15 min at 4°C, samples were stored at -80°C. Nicotine analyses were performed at the University of Michigan in the laboratory of M. Hariharan, using high-performance liquid chromatography (Hariharan, Van Noord, & Greden, 1988). The method has an inter-assay CV of 8.0%, an intraassay CV of 6.6%, and a lower limit of detectability of 1 ng/ml for nicotine in plasma.

RESULTS

Figure 2 shows the time course for plasma nicotine values for the different conditions. Mean change ($\pm SD$) in plasma nicotine between Minutes 0 and 10 was -1.2 ± 1.9 ng/ml for the zero-nicotine session, 10.0 ± 7.0 ng/ml for the medium-nicotine session, and 28.0 ± 13.1 , 25.4 ± 7.8 , and 26.0 ± 17.3 ng/ml for the three high-nicotine sessions. Peak levels occurred immediately after airbag smoking (10 min after session start) in the first high-nicotine condition but 5 min later for 5 of the 9 subjects in the medium-nicotine condition (15 min after session start).

Subexperiment 1: Parametric dosing. Significant differences in nicotine change scores were detected for the zero-, medium-, and first high-nicotine sessions [$F(2,16) = 25.56, p = .0000$]. A priori analyses showed

Figure 2. Mean plasma nicotine as a function of time. (Plasma nicotine was assayed for Timepoints 0 and 10 (minutes) for the zero-nicotine session, for Timepoints 0, 10, and 15 for the first and third high-nicotine sessions, and for Timepoints 0, 10, 15, 20, 25, and 30 for the medium-nicotine and the second high-nicotine sessions; the second rather than the first high-nicotine session was chosen for the full time-course assay because of missing data at one of the later timepoints for the first session.)

significant differences for all possible pairwise comparisons (medium vs. zero, $p < .05$; medium vs. high and zero vs. high, $p < .001$). Between-subject CVs for the medium- and first high-nicotine sessions were 70% and 47%, respectively, for the plasma nicotine increment from Minute 0 to Minute 10 (immediately after smoking). Between-subject CVs for base-to-peak increments for the medium- and first high-nicotine sessions were 42% and 47%, respectively.

Table 1 shows change scores (Minute 10 - Minute 0) for the zero-, medium-, and first high-nicotine sessions for physiological and subjective variables. A priori multiple comparisons revealed decreased craving and withdrawal, elevated heart rate, and lowered skin temperature, comparing the high-nicotine condition and one or both of the other two conditions. No significant differences between the zero-nicotine and the medium-nicotine condition were detected for any of these variables.

Subexperiment 2: Repeated dosing. No significant differences in nicotine change scores were detected among the three high-nicotine sessions [$F(2,16) = 0.18, n.s.$]. (Subjective and physiological data for the second and third high-nicotine sessions resembled those collected on the first.) Between-subject CVs for the first, second, and third high-nicotine days were 47%, 31%, and 66%, respectively, for a mean of 48%. Within-subject CVs (comparing nicotine boost over three sessions for a given subject) were, in order of increasing size, 8%, 16%, 18%, 21%, 24%, 31%, 43%, 44%, and 66%, for a mean within-subject CV of 30%. Spearman correlation coefficients for the three high-nicotine sessions are $+ .51$ (n.s.), comparing the first session with the second; $+ .72$ ($p = .03$), comparing the first with the third; and $+ .62$ ($p = .07$), comparing the second with the third.

Table 1
Change Scores (Minute 10 – Minute 0) for Subjective and Physiological Variables
(Mean $\pm$ Standard Deviation)

Variables	Zero-Nicotine	Medium-Nicotine	High-Nicotine, Day 1
Subjective			
Craving (arbitrary units)	-20.1 $\pm$ 12.9	-19.3 $\pm$ 20.0 ^{a*}	-31.3 $\pm$ 14.7 ^{a*}
Withdrawal (arbitrary units)	-12.8 $\pm$ 9.8 ^{a*}	-2.8 $\pm$ 9.8 ^{b†}	-24.9 $\pm$ 13.5 ^{a*b†}
Physiological			
Heart rate (beats/minute)	2.7 $\pm$ 3.3 ^{a†}	2.2 $\pm$ 2.8 ^{b†}	9.0 $\pm$ 6.9 ^{a†b†}
Temp ($^{\circ}$ C)	-0.33 $\pm$ 0.8 ^{a*}	-0.23 $\pm$ 0.8 ^{b†}	-0.98 $\pm$ 1.0 ^{a*b†}

Note—Lowercase letters indicate the pairwise comparisons for which trends or significant differences were observed. *trend ($p < .10$). [†] $p < .05$. [‡] $p < .01$.

Since weight range for the 9 subjects was nearly 50 kg, an attempt was made to determine whether the heavier subjects achieved smaller boosts in plasma nicotine by virtue of having received lower doses of smoke per kilogram of body weight. Pearson correlation coefficients of weight with nicotine boost for each of the three high-nicotine sessions were $-.20$ (n.s.), $+.01$ (n.s.), and $-.12$ (n.s.), respectively. The 3 lightest subjects (mean weight 63 kg) showed a mean plasma nicotine boost of 26.3 ng/ml over the three high-nicotine sessions; the intermediate 3 (mean weight 74 kg) showed a mean boost of 29.2 ng/ml; and the 3 heaviest subjects (mean weight 96 kg) showed a mean boost of 22.8 ng/ml.

DISCUSSION

Plasma nicotine levels resulting from the airbag smoking procedure reached a peak shortly after completion of smoking and were followed by a gradual decay; both the levels and the pattern were comparable to that observed for ordinary cigarette smoking (see, e.g., O. F. Pomerleau, C. S. Pomerleau, & Rose, in press; Russell, Jarvis, Feyerabend, & Ferno, 1983). The significance of the 5-min delay in peak nicotine at the medium dose level is not immediately obvious, though differences in distribution kinetics for different dose levels is a possibility. The physiological and subjective data show clear-cut nicotine-related responses for the high-nicotine condition; findings for the medium-nicotine and the zero-nicotine conditions were not statistically different from one another, possibly because of secondary reinforcement from airway stimulation by smoke from the zero nicotine cigarette (see Rose, 1988). At debriefing, the subjects stated that the airbag procedure was generally pleasurable and that the effects were similar to ordinary smoking.

Among the shortcomings of the approach, we note several factors that could have contributed to individual variability in peak plasma nicotine concentrations. First, the rate at which subjects inhaled was not strictly controlled. Variations in inhalation rate could have affected regional airway deposition and subsequent rate of nicotine absorption. Second, because subjects' nostrils were not pinched shut, additional air could have been taken in through the nose during inhalation, causing somewhat more than 1 liter of air to enter the lungs. Although our

procedure ensured that at least 1 liter of air was inhaled, producing nearly maximal deposition of nicotine via a 3-sec breathhold, some differences in absorption may have resulted from variability in the volume of air inhaled.

From the perspective of average dose control (examining the 9 subjects as a group), the airbag procedure produced a statistically significant graded response, with the medium dose slightly less than halfway between the zero- and high-nicotine doses. Using base-to-peak means, dose response was linear, with the 30-cc dose of high-nicotine smoke (the medium-nicotine condition) producing a mean plasma nicotine boost of approximately half those produced by the 60-cc doses of high-nicotine smoke (the high-nicotine conditions). Investigation with additional dose levels will be needed to ascertain whether this method is sufficiently precise to differentiate more than the three administration levels used in the present study. Repeated dosing at the high-nicotine level showed moderate consistency, with mean plasma nicotine values for the group differing only by a few nanograms/milliliter across sessions. Although the 3 subjects who weighed the most showed slightly smaller nicotine boosts than the 3 lightest, our overall findings regarding body weight were inconclusive, and it is not clear that attempting to adjust dose to unit body weight would produce better results.

The computerized topography control procedure employed by Zacny et al. (1987), which also represents an attempt to administer controlled doses of nicotine via cigarette smoke to minimally deprived smokers, provides a comparison for the present method. In that study, 10 subjects took eight puffs of smoke at 60-sec intervals, with puff volume varying from 15 cc to 60 cc in four steps. A graded plasma nicotine boost was obtained, going from 4.6, 7.5, 11.8, and 16.2 ng/ml. CVs for the different dose levels varied from 39% to 60% (computed from data supplied by Zacny, personal communication), which is similar to that obtained in the present study. Since Zacny et al. did not carry out repeated dosing at a given level, it is not possible to examine dosing consistency in their procedure.

The airbag procedure described here offers a promising method for administering predetermined amounts of nicotine in smoke. With respect to repeated dosing, the airbag procedure is more reliable than uncontrolled smoking or paced puffing (C. S. Pomerleau, Majchrzak, &

O. F. Pomerleau, 1989b), though clearly less reliable than intravenous nicotine infusion (O. F. Pomerleau, C. S. Pomerleau, & Rose, in press). Although topography-control techniques do show potential, evaluation procedures will need to be standardized and testing conducted under different smoking conditions, including various deprivation levels and situational demands, and involving smokers of differing degrees of dependence, before their adequacy for controlled dosing can be considered established. At this point, the outstanding features of our approach are safety and apparent comparability to usual smoking. For a more complete discussion of these and related issues, see a recent evaluation of nicotine dose-control methodologies by O. F. Pomerleau, C. S. Pomerleau, and Rose (in press).

Between-subject variability was considerably higher than within-subject variability, suggesting that each subject obtained a characteristic nicotine boost. The extremely rapid absorption of nicotine from cigarette smoke in the lungs permits variations in the rate of distribution of nicotine from blood into body organs to affect nicotine levels measured shortly after the dosing procedure (Porchet, Benowitz, & Sheiner, 1987). For example, in the study by Feyereabend et al. (1985), nicotine levels were measured within 2 min of a rapid (1-min) injection, and plasma nicotine increments showed CVs of 61% for the first injection. There was similarly large between-subject variation in initial distributional volume (CVs of 57%) but much smaller variability in distributional half-life (CVs of 13%). Thus, variations in nicotine pharmacokinetics may cause individual differences in peak nicotine, even though the dose may have been perfectly controlled (Benowitz, Jacob, Jones, & Rosenberg, 1982). Further refinements in the development of reliable fixed dosing techniques may well require determination of individual differences in nicotine pharmacokinetics in order to adjust nicotine dosage appropriately.

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