Pharmacodynamics of Acute Tolerance to Multiple Nicotinic Effects in Humans

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ABSTRACT

Tolerance is an important determinant of addiction as well as therapeutic and/or toxic effects of drugs. The development of acute tolerance to various effects of nicotine was studied in nine healthy smokers who were abstaining from tobacco. Nicotine was infused rapidly to reach a concentration of about 25 ng/ml, followed by a computer-controlled infusion to maintain that concentration. A novel semiparametric model of nicotine effects and tolerance was developed. Tolerance to various effects of nicotine (increases in heart rate, blood pressure, plasma epinephrine and energy expenditure) occurred within the range of nicotine levels found in smokers. However, the rate of tolerance development varied considerably. The half-lives of tolerance ranged from 3.5 min for the increase in energy expenditure to 70 min for systolic blood pressure. There was no apparent tolerance to the effects on free fatty acid concentrations, which reflects lipolysis. Differences in the pharmacodynamics of tolerance may reflect differences in rate of desensitization of various subtypes of nicotinic receptors and/or differences in mechanisms of tolerance for various nicotinic effects.

Acute tolerance develops to many of the effects of drugs of abuse, including nicotine. The mechanism of acute tolerance to nicotine seems to involve primarily desensitization of nicotinic cholinergic receptors (Wonnacott, 1990). Acute tolerance to effects of nicotine has been well characterized for electrophysiologic responses in cultured cells, release of acetylcholine, dopamine and rubidium from brain synaptosomes, and blood pressure, ACTH, corticosterone, prolactin release, locomotor activity and body temperature depression in intact animals (Vibat et al., 1995; Grady et al., 1994; Rowell and Hillebrand, 1994; Marks et al., 1993, 1996; Caggiula et al., 1991; Hulihan-Giblin et al., 1990a, b; Sharp and Beyer, 1986; Aceto et al., 1986). In addition to receptor desensitization, homeostatic responses may also contribute to acute tolerance. For example, glucocorticoid release with negative feedback is partially responsible for development of tolerance to ACTH secretion produced by nicotine (Pauly et al., 1992).

Nicotinic receptors are present in varying concentrations in different parts of the brain, and nicotine receptors modulate release of different neurotransmitters, including dopamine, norepinephrine, acetylcholine, serotonin, glutamate, β-endorphin and others (Clarke et al., 1985; McGehee et al., 1995; Yu and Wecker, 1994). Thus, it is not surprising that in an intact organism nicotine has diverse effects on various organ systems, effects which may depend on the ongoing behavior of the organism.

Brain nicotinic receptors are composed of alpha and beta subunits, which may be combined to form various subtypes of receptors (McGehee and Role, 1995). These subtypes have different nicotinic agonist binding characteristics and different electrophysiologic characteristics. Responses to different nicotinic receptor subtypes desensitize at different rates. For example, alpha-7-containing receptors desensitize more rapidly than alpha-3/β-2 or alpha-2/β-2 receptors, which desensitize more rapidly than alpha-4/β-2 receptors (Alkondon and Albuquerque, 1993; Vibat et al., 1995). The overall reduction in response to nicotine has been shown to be greater for alpha-4/β-2 than for alpha-3/β-2 or alpha-2/β-2 receptors (Vibat et al., 1995). Thus, the extent and rate of development of tolerance to various effects of nicotine might be greater for alpha-4/β-2 than for alpha-3/β-2 or alpha-2/β-2 receptors (Vibat et al., 1995).

ABBREVIATIONS: V1, volume of distribution of central compartment; V2, volume of distribution of peripheral compartment; Vss, steady-state volume of distribution; CL, total plasma clearance; t1/2a, distribution half-life; t1/2β, elimination half-life; kαβ, intercompartmental transfer rate constants; Cω, concentration at the hypothetical effect site; Kω, rate constant of exit from the effect site; Cm, concentration of the hypothetical antagonist metabolite; Kωm, exit constant from the metabolite compartment; E, effect compartment; ACTH, corticotropin.
nicotine is expected to differ based on different receptor subtypes and different types of homeostatic responses. This has been seen in animals, where a different extent and duration of tolerance has been observed for different nicotinic effects in rats during chronic (12-day) nicotine dosing (Marks et al., 1985).

Although much research has been conducted on the kinetics of receptor desensitization with in vitro responses, very little has been done on quantitatively studying the extent and rate of tolerance in relation to nicotine concentrations for different responses in intact organisms, including people. Because nicotine responses are complex, studies of tolerance cannot be readily extrapolated from animals to people. To understand the pharmacology of nicotine as it is relevant to addiction, one must specifically study tolerance in humans.

We and others have been studying the phenomenon of acute tolerance development to nicotinic responses in humans. Acute tolerance to subjective effects, heart rate increase and increased metabolic rate has been described (Porchet et al., 1988; Arcavi et al., 1994; Perkins et al., 1993). Tolerance is important because tolerance to psychoactive effects contributes to nicotine addiction. Acute tolerance influences how reinforcing nicotine self-administration is at various times of the day during regular tobacco use, and it may determine temporal patterns of tobacco use (Benowitz, 1990). Acute tolerance to cardiovascular and metabolic effects may have an impact on the potential adverse effects of nicotine on the cardiovascular system and the effects of nicotine on body weight (Arcavi et al., 1994). As in animals, the rate and extent of the development of tolerance appears to vary for different nicotinic responses. For example, Arcavi et al. (1994) found a different pattern of development of tolerance to heart rate acceleration and metabolic rate when comparing light and heavy smokers.

In the present study, we have attempted to characterize in a quantitative fashion the development of tolerance to several nicotinic responses in people. To optimize the quantitative procedure, we used an experimental paradigm in which nicotine was infused rapidly to an expected steady-state level, followed by a computer-controlled infusion to maintain that steady-state concentration. This dosing paradigm results in a plateau concentration during which the effects of the drug may be observed to diminish over time, thereby giving information on the rate of development of tolerance. A similar technique has been used by other laboratories in studying the effects of cocaine (Ambre et al., 1988).

We attempted to estimate the pharmacodynamics of tolerance by use of a model developed previously by Porchet et al. (1988) in this laboratory. The Porchet model was based on an experimental paradigm of paired intravenous infusions of nicotine, spaced at different intervals of time. In the present study, we found that the Porchet model was inadequate to characterize the development of tolerance to some nicotinic responses, and therefore we developed a more general, semiparametric pharmacodynamic model of tolerance development as well.

Methods

Subjects. Nine subjects, eight men and one woman, 26 to 62 years of age (average, 42 years), who were habitual cigarette smokers, were recruited by newspaper advertisements. All were healthy based on medical examination, blood chemistries and electrocardiogram. They smoked an average of 28 cigarettes per day (range, 25–40 cigarettes). Their Fagerström dependence score averaged 7.0 (range, 6–9) (Fagerström, 1978). The average screening plasma cotinine concentration was 277 ng/ml (range, 185–364 ng/ml).

Experimental protocol. Subjects were hospitalized in the General Clinical Research Center at San Francisco General Hospital for 3 days. No smoking was allowed from 9:00 P.M. each evening until completion of the study on the following day. On the mornings of days 2 and 3, after an overnight fast, subjects received an intravenous infusion of nicotine or saline (placebo). The sequence of infusions was counterbalanced. Subjects were blind to the treatment. Nicotine was infused via a computer-controlled infusion pump. The software to control the pump, developed by Schafer, is based on a two-compartment body model (Schafer and Gregg, 1992). Population pharmacokinetic data observed in a prior study in our laboratory were used. These parameters were: $V_1 = 1.14$ l/kg, $k_{12} = 0.023$ min$^{-1}$, $k_{10} = 0.016$ min$^{-1}$, $k_{21} = 0.013$ min$^{-1}$, $\alpha = 0.048$ min$^{-1}$ and $\beta = 0.0044$ min$^{-1}$.

The infusion was programmed to reach a target plasma nicotine concentration of 25 ng/ml, similar to that seen in the venous blood of typical cigarette smokers (Benowitz, 1988). The time from initiation of infusion to target plasma nicotine concentration was set at 2 min for three subjects, 3 min for three subjects and 5 min for three subjects. Different rates of loading were chosen to study the influence of rate of dosing and resultant effects. However, as no differences were seen based on rate of loading in the data analysis, data from all subjects were combined for pharmacokinetic-pharmacodynamic modeling. The maintenance infusion was continued for a total of 180 min. This dosing paradigm differs from that experienced by smokers, who take in nicotine intermittently, but was necessary to optimize the pharmacodynamic analysis.

Plasma samples were obtained from an indwelling venous catheter during infusion and for 60 min after the end of infusion for measurement of nicotine and free fatty acids. The latter is a measure of lipolysis, a consequence of sympathetic activation by nicotine. Plasma catecholamine measurements were made for 20 min before and 90 min during the nicotine infusion. Sampling was stopped at 90 min when subjects were allowed to get up to urinate. Blood pressure and heart rate were measured at frequent intervals by an automated blood pressure recording machine (Dinamap, Critikon, Inc., Tampa, FL). In seven subjects, energy expenditure (metabolic rate) was measured continuously by indirect calorimetry, as described below, for 90 min before and for the first 30 min during the infusion. (Energy expenditure measurements were not made in the two subjects with 2 min loading to achieve target plasma nicotine concentrations.)

Concentrations of nicotine and cotinine were determined by gas chromatography with nitrogen-phosphorus detection, with 5-methylnicotine and 1-methyl-5-(2-pyridyl)-pyrroline-2-one (‘ortho-cotinine’) as internal standards (Jacob et al., 1981). This method has been modified for simultaneous extraction of nicotine and cotinine and determination by use of capillary gas chromatography.

Plasma catecholamines were assayed by high-performance liquid chromatography with use of an electrochemical detector (Higa et al., 1977). The limit of quantitation for epinephrine and norepinephrine was 12.5 pg/ml. Plasma concentrations of nonesterified fatty acids were measured by an enzymatic colorimetric method with kits obtained from Waco Chemicals USA, Inc. (Dallas, TX).

Energy expenditure and respiratory quotient were measured by indirect calorimetry with the use of a Delta Trac Metabolic Monitor (Sensor Medics Corp., Yorba Linda, CA) with a ventilated hood system for collecting expired gases. During indirect calorimetry, the head of the patient was covered with a transparent canopy through which there is a constant fresh air flow of 40 l/min. Energy expenditure was determined by measuring oxygen consumption and carbon dioxide production. A paramagnetic oxygen sensor was used to measure oxygen consumption. A continuous measurement of the
carbon dioxide concentration in the expired air allowed computation of the amount of carbon dioxide eliminated by the patient. The calorimetry system was calibrated before each measurement. Data were analyzed continuously on-line, and a value for energy expenditure displayed as averaged 1-min epochs.

Because of the canopy and the necessity to minimize muscular activity (such as writing) during the energy expenditure measurement, subjective responses could not be recorded during the first 30 min of infusion. A report on symptoms experienced was requested retrospectively, after the energy expenditure measurement was complete.

**Data analysis.** For all pharmacokinetic and pharmacodynamic analyses, we used mixed effect models with the computer program NONMEM (Beal and Sheiner, 1992). For all pharmacokinetic and pharmacodynamic fits, an additive plus proportional intraindividual error model was used. The 95% confidence intervals reported for the pharmacodynamic parameters were obtained by means of a likelihood ratio profile (Bates and Watts, 1988).

The pharmacokinetic-pharmacodynamic analysis was performed in several steps. First, the convolution of a two-compartmental model with the subject’s infusion rate administered by the computer-controlled infusion pump was fitted to the nicotine plasma concentration measurements. Thereafter, for each subject, empirical Bayes estimates for all estimated parameters were obtained. The corresponding mean estimates are reported under “Results.” In the pharmacokinetic analysis, predictions for nicotine plasma concentrations for each subject were calculated with the subject’s empirical Bayes parameter estimates and the subject’s input rate function.

The response measures were observed to change over time during placebo infusions. To account for these changes, a model for placebo response was included in the analysis. We analyzed the placebo data of each pharmacodynamic effect by use of the following model, which describes the i-th base-line datum from the j-th subject (Bij) as a function of time (t):

\[ B_{ij} = s_i(t_{ij}) + \eta_{ij} \]

where \( t_{ij} \) is the time of the \( ij \)-th datum, \( s_i(t_{ij}) \) is a nonparametric function, a natural cubic spline (DeBoor, 1978) and \( \eta_{ij} \) are assumed normally distributed with mean zero and variance to be estimated and represent the subject’s shifts of base line. The breakpoints of the spline are positioned at the quantiles of the \( t_i \) (Verotta, 1993; Stone and Koo, 1986; Hastie and Tibshirani, 1990). The number of parameters for the spline function was selected by use of the Akaike criterion (Akaike, 1974). Empirical Bayes estimates (\( \hat{B}_{ij} \)) for each subject’s base line were obtained. The base line for each subject and each effect was fixed to these values during the pharmacodynamic analysis.

Thereafter, each pharmacodynamic effect was analyzed separately, with the two models shown schematically in figure 1. Both pharmacokinetic and pharmacodynamic fits are interindividual random effect parameters with mean zero and variance to be estimated. This model is similar to the model used by Porchet et al. (1988) to describe heart rate as a function of nicotine concentration. We added an effect compartment to the model instead of using the concentration in the central (pharmacokinetic) compartment because this resulted in a better fit of the observed data for some of the effects.
For model selection, we compared the plots of the predicted response versus the data for the different models and used the Akaike criterion (Akaike, 1974). The Akaike criterion requires a decrease of the −2 log likelihood (L), which represents a measure of the goodness of fit for the corresponding model, of two points or more per parameter to select the larger over the smaller model, i.e., eight points or more for selecting model 2a over model 1, two points or more for selecting 2b over 1, and six points for selecting 2a over 2b. Models 1 and 2c have the same number of parameters.

Results

The infusions of nicotine were well tolerated. All subjects had subjective responses to the nicotine infusion. The most common symptom was dizziness. Three subjects reported nausea or an unpleasant sensation in the stomach; two reported tingling in the extremities; and two subjects became anxious. Subjective references peaked at the end of the loading infusion (i.e., in 2–5 min) and resolved within 10 min. Most subjects reported no symptoms at all for the remainder of the study. One subject reported a headache that persisted until 90 min after the nicotine infusion was discontinued.

Figure 2 compares the subjects’ observed plasma nicotine concentrations with the average predictions from the pharmacokinetic model. The plasma nicotine levels were seen to overshoot the target, reaching about 30 ng/ml, but nicotine levels rapidly fell thereafter and remained close to the target 25 ng/ml for the duration of the infusion.

Table 1 provides the average pharmacokinetic parameters obtained from the subjects’ empirical Bayes estimates. These parameters differed somewhat from those used to program the infusion, most likely because the data used to program the infusion were based on constant rate 30-min intravenous infusions in a previous study.

Nicotine increased heart rate, systolic and diastolic blood pressure, plasma epinephrine and free fatty acid concentrations, and it increased energy expenditure (metabolic rate) as shown in the left-hand panels of figures 3 to 5. Long dashed lines in these figures demonstrate the fits obtained by the use of two different pharmacodynamic models. The fits to the placebo data are shown in the solid lines. The short dashed lines show the averaged plasma nicotine concentration in the central compartment as predicted by the pharmacokinetic model (same as short dashed line in fig. 2).

The middle panel shows the relationship between the effect compartment concentration and response, providing a pictorial representation of the pharmacodynamic model without the development of tolerance. The two effect models are shown by medium and long dashed lines. The right panels depict the relationship between concentration in the hypothetical metabolite compartment and the degree to which a particular effect would be diminished compared with the effect that would have been produced in the absence of tolerance. The right-hand panels provide a pictorial of the tolerance functions for the two different models.

Table 2 shows the difference between the minimum of the objective function of models 1 and 2 and the parameter estimates for \( k_{ao} \), \( k_{mo} \) and \( C_{m50} \) for models 1 and 2. We also report 95% confidence intervals for the selected model. In model 2, no confidence interval for \( C_{m50} \) was reported, because the parameter was not part of the model itself, which made it infeasible to obtain a likelihood ratio profile.

For heart rate, model 2a produced a slightly improved fit compared with model 1, whereas model 2b did not improve the fit compared with model 1. This indicates that it was not the change of the tolerance function which caused the improvement of the fit. The main difference (fig. 3) between models 1 and 2 is that in the latter the effect function stops increasing if \( C_{o} \) rises above 20 ng/ml. Model 2a fits the data at early times after drug administration slightly better than model 1 (see fig. 3, upper panels). At later times, the fits are very similar.

For diastolic, systolic and mean blood pressure, model 2a does not improve the fit compared with model 1 (see fig. 3). The estimate of \( k_{mo} \) is quite large. Indeed, we can substitute nicotine concentration in the central compartment for concentration in the effect compartment without change of objective function. In systolic and mean arterial blood pressure, the parameters of the model are not estimated very precisely. For the diastolic blood pressure response, figure 3 shows that models 1 and 2 somewhat overpredict the early responses.

The pharmacodynamic effect of nicotine on energy expenditure is described better by model 2a than by model 1. This can also clearly be seen when comparing both fits in figure 5 (upper panels). Model 2b yields a better fit than model 1 (difference in −2 log likelihood (ΔL) is 40.8), but a worse fit than model 2a (ΔL = 26), which indicated that both the change in tolerance and effect model contribute to the improvement of the fit. The estimate of \( k_{mo} \) is small for model 1,

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**Table 1**

<table>
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<tr>
<th>Parameter</th>
<th>Units</th>
<th>Estimate</th>
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<td>0.0260</td>
<td>(0.001)</td>
</tr>
<tr>
<td>( k_{12} )</td>
<td>min(^{-1} )</td>
<td>0.0684</td>
<td>(0.001)</td>
</tr>
<tr>
<td>( k_{21} )</td>
<td>min(^{-1} )</td>
<td>0.0363</td>
<td>(0.0003)</td>
</tr>
<tr>
<td>( V_1 )</td>
<td>l kg(^{-1} )</td>
<td>0.867</td>
<td>(0.032)</td>
</tr>
<tr>
<td>( CL )</td>
<td>l min(^{-1} ) kg(^{-1} )</td>
<td>0.0210</td>
<td>(0.0006)</td>
</tr>
<tr>
<td>( Vss )</td>
<td>l kg(^{-1} )</td>
<td>2.48</td>
<td>(0.08)</td>
</tr>
<tr>
<td>( t_{1/2a} )</td>
<td>min</td>
<td>5.66</td>
<td>(0.06)</td>
</tr>
<tr>
<td>( t_{1/2b} )</td>
<td>min</td>
<td>98.6</td>
<td>(3.4)</td>
</tr>
</tbody>
</table>

The rate constants are described in figure 1.

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**Fig. 2.** Plasma nicotine concentrations (dots) and average concentration (dotted line) predicted by the pharmacokinetic model during and after the nicotine infusion.
the corresponding half-life is more than 1 day. But, despite this long half-life, relevant tolerance is reached during the experiment because the estimate of $C_{\text{m50}}$ is also very small, which indicates that minimal amounts of hypothetical metabolite cause almost complete tolerance.

For plasma epinephrine concentrations, the response in one of the subjects was 10 times as high as the response in any of the other subjects, which caused problems in fitting the data. To keep this subject in the analysis, we introduced an additional parameter which scaled this subject’s response compared with the others. Because model 1 estimates a long half-life for tolerance development (340 min) and the objective function for model 2c is slightly lower, we prefer model 2c over model 1. Figure 4 (lower panels) shows the corresponding fits. However, the only conclusions these data allow us to make for the epinephrine response to nicotine are that 1) nicotine increases plasma epinephrine concentration, 2) tolerance occurs and 3) after 90 min, tolerance has reduced drug effect to 20% of the initial effect. We cannot determine whether, after 90 min, tolerance increases further, and how much of the drug effect remains when tolerance has completely developed.
For the plasma free fatty acids, it appears from the raw data (fig. 5, lower panels) that there is no relevant tolerance development during the experiment. One observes a slight increase of the plasma free fatty acid concentration over the entire duration of drug administration. Correspondingly, model 1 yields a very high estimate for $C_{m50}$ and a very small estimate of $k_{mo}$, practically eliminating tolerance from the model. In model 2, the tolerance part, $s_3(C_{m}(t_{ij}))$, can be removed without significant change of the fit.

For plasma norepinephrine, no consistent response was observed for the eight subjects studied. Consequently, no fit of the data was attempted.

**Discussion**

Our paper provides novel information in two respects. First, the pharmacodynamic modeling represents an advance, with greater generalizability than our prior attempts at model tolerance. Second, this is the first time the pharmacodynamics of tolerance to multiple pharmacologic responses observed simultaneously in people has been studied.

When we use the parametric tolerance model proposed by Porchet *et al.* (1988), we make stringent assumptions about the shape of the tolerance and the effect functions. For some responses, these assumptions appear to be appropriate, as for example for the blood pressure responses. However, sometimes the assumptions are incorrect, resulting in strange parameter estimates. For example, for energy expenditure and epinephrine responses, we obtained unreasonably low estimates for $C_{m50}$ combined with small estimates for $k_{mo}$, which indicated very slow tolerance development. If our modeling tools had been limited to parametric functions at that point, we would have had to guess what part of our model might be wrongly specified by trying additional parametric models. Such a search for the correct structural parametric model can be difficult or even unsuccessful. In contrast, the nonparametric functions we used allowed us to estimate the appropriate effect and...
tolerance functions directly by avoiding unnecessarily stringent assumptions about their shape.

Because the explorative tool we propose uses a flexible function to describe both the tolerance and the effect models, we must be concerned about the identifiability of these two functions. Because of this concern, we tested the tolerance part of the semiparametric model separately from the flexible effect model by use of model 2b (the more flexible tolerance model combined with a linear effect model). For the energy expenditure data, we clearly saw that we needed both parts of our explorative tool to obtain an adequate fit, which indicated that the model is identifiable if adequate data are available. For the heart rate response, we found that the more flexible effect function was responsible for the improvement of the fit. In the case of epinephrine, however, we realized that there were not enough data available to determine the shape of the tolerance and effect functions reliably and, consequently, reduced the model to two linear functions.

For the diastolic (and mean) blood pressure responses, we noticed a misfit of the population model in the early part of our experiment for model 1 and model 2a. In contrast, for both models the individual fits do not show the same misfit (not shown). Also, the mean of the individual random effect \( \eta_{ij} \), which is assumed to be zero, is \(-0.322\). This is quite different from zero considering that the estimate of the standard deviation of \( \eta_{ij} \) is 0.584. Based on similar observations in other population analyses (Youngs et al., submitted for publication), we suspect that the misfit of the population model is caused by some misspecification in our model, probably a misspecification of the variance model. The model we use for interindividual variability implies that the individual's responses differ only by a scale in direction of the y-axis, but allows no differences in the time course of the response between individuals. There is no reason why interindividual differences should not be present for the time course of effect or tolerance development; however, we are not able to successfully include such variability in our population model.

Tolerance to the effects of nicotine has been examined extensively in vitro and in animals, and appears to involve at least two mechanisms. Acute tolerance appears to involve shifting of nicotinic cholinergic receptors from an active to a desensitized inactive state (Wonnacott, 1990). Chronic tolerance may be associated with an increase in nicotinic cholinergic receptor number (Aceto et al., 1986; Marks et al., 1987). A likely explanation for the latter is that nicotine acts initially as an agonist, but then binds with high affinity to the receptor, resulting in persistent inactivation which, in turn, stimulates production of more receptors (Wonnacott, 1990). Tolerance may also involve homeostatic responses to nicotine-induced physiologic perturbations, such as the negative feedback of glucocorticoid release on nicotine-mediated ACTH secretion (Pauly et al., 1992).

In people, tolerance to subjective effects, heart rate acceleration and increased energy expenditure has been described (Porchet et al., 1988; Perkins et al., 1993; Arcavi et al., 1994). But only heart rate acceleration effects have been modeled (Porchet et al., 1988). The results of the present study allow us to compare the pharmacodynamics of tolerance development for several responses. It should be noted that the subjective and other central nervous effects of nicotine are of most interest with respect to addiction. Unfortunately, we were unable to record subjective effects during the first 30 min of nicotine infusion (beyond which there were no subjective effects), and we have at this time no other quantitative measures of central nervous system effects that we were able to model. Therefore, our study focuses on cardiovascular, hormonal and metabolic effects of nicotine.

The pharmacodynamic parameters of most interest are the \( C_{m50} \) values and the half-life of development of tolerance. The \( C_{m50} \) value represents the concentrations at which the effect is 50% of the effect that would have occurred in the absence of tolerance. The \( C_{m50} \) concentrations for the various responses are close, ranging from 6.7 ng/ml (for systolic blood pressure) to 12.5 ng/ml (for epinephrine response). The \( C_{m50} \) for heart rate estimated in this study (8.9 ng/ml) is very similar to that estimated previously by Porchet et al. (7.7 ng/ml) (1988). The confidence intervals are such that these estimates are not significantly different. These concentrations are consistent with the \( EC_{50} \) concentration reported for nicotine in desensitizing nicotine-mediated rubidium efflux from mouse synaptosomes and in the low range of nicotine levels found in smokers (Marks et al., 1996). Thus, considerable tolerance will be expected for all these responses in most smokers.

For the parametric model (model 1), the effect after full development of tolerance is computed as:

\[
\frac{1}{1 + \frac{C}{C_{m50}}}
\]

For the semiparametric model (model 2), the number can be read from the rightmost panels of figures 3 to 5. Thus, at a steady state nicotine concentration of 25 ng/ml, the resultant effects will be one-fifth to one-third of the effect that would have been present had tolerance not developed.

The other parameter of particular interest is \( k_{mo} \) or taken as \( \ln2/k_{mo} \), the half-life of development of tolerance (assuming an instantaneous plateau of nicotine level). The half-life of tolerance was quite variable for different measures, ranging from 70 min for systolic blood pressure to 3.5 min for energy expenditure. Thus, tolerance to systolic blood pressure develops relatively slowly, requiring several hours for maximal tolerance to develop, whereas nearly complete tolerance to the increase in energy expenditure is expected within 15 min. Comparing estimates from two different studies with the same parametric model, the estimate for \( k_{mo} \) for the heart rate response in the present study (0.032 min\(^{-1}\)) was similar to that estimated by Porchet (0.020 min\(^{-1}\)) (Porchet et al., 1988). The corresponding half-lives for tolerance were 21 min vs. 35 min. Both estimates indicate relatively rapid development of tolerance to heart rate acceleration.

That the rates of development of tolerance for heart rate, blood pressure and plasma epinephrine responses are reasonably similar is consistent with the idea that all are sympathetic neural responses and suggest that epinephrine may contribute to these responses. The extremely rapid development of tolerance to the increase in metabolic rate found in the present study suggests that this effect is mediated by a mechanism other than generalized sympathetic neural activation. Given the rapid rate of desensitization, the possibility that metabolic rate represents a nicotine effect on a rapidly
desensitizing receptor subtype different from that mediating the cardiovascular effects must be entertained.

We did not find a consistent increase in plasma norepinephrine concentrations. Plasma norepinephrine concentration represents a spillover after synaptic release of norepinephrine, and is not a sensitive measure of sympathetic neural activation. In some of our previous studies, cigarette smoking was shown to increase urinary epinephrine excretion more than norepinephrine excretion, consistent with the findings in the present study (Benowitz et al., 1983; Benowitz and Jacob, 1990).

Of considerable mechanistic interest is the observation that the free fatty acid response did not demonstrate tolerance in the current paradigm. Nicotine releases free fatty acids from triglycerides in adipose tissue, presumably via an adrenergic mechanism (Ilebekk et al., 1975). Free fatty acids are rapidly cleared from plasma so, in general, fatty acid levels in the blood reflect release rate (Havel et al., 1964). The observation of Hellerstein et al. (1994) that cigarette smoking increases steady-state serum free fatty acid concentrations and increases the release rate of fatty acids to the same degree indicates that smoking (and presumably nicotine) is not affecting the clearance of fatty acids. Therefore, we can assume that the plasma and fatty acid concentrations observed in our study reflect the actions of nicotine in adipose tissue. The lack of development of tolerance in our study is consistent with the earlier observation that fatty acid flux was persistently elevated over 3 hr of smoking (Hellerstein et al., 1994). The discordance between the development of tolerance to the fatty acid response compared with the plasma epinephrine and metabolic rate responses has mechanistic implications. Since epinephrine levels fall to near baseline within 90 min, it is unlikely that epinephrine is responsible for the sustained lipolysis seen during 3 hr of nicotine infusion. This suggests that the mechanism for nicotine-induced lipolysis is not systemic catecholamine release but rather local release of norepinephrine. Of possible relevance in this regard are studies of neurotransmitter release from brain slices showing no tolerance to norepinephrine release, while tolerance did develop to dopamine and serotonin release (Yu and Wecker, 1994).

Our results are also relevant to understanding the biochemical mechanisms of the nicotine effect on metabolic rate. The observation that fatty acid flux remains elevated whereas metabolic rate returns to normal within a few minutes indicates that lipolysis with futile cycling of free fatty acids is not the mechanism for the nicotine effect on metabolic rate. Futele cycling has been suggested to be the link between sympathetic nervous system stimulation and the increase in metabolic rate (Wolfe et al., 1987), but this appears not to be the case for nicotine.

In summary, we present a more general variant of a pharmacokinetic-pharmacodynamic tolerance model previously. Relaxing the stringent assumptions about the shape of the tolerance effect functions allows us to compare the pharmacodynamics of tolerance to several responses to nicotine, differences among which may indicate differences in subtypes of nicotinic receptors involved in responses and/or mechanisms of development of tolerance. Such methods should prove useful in studying factors that influence the development or regression of tolerance to nicotine and other drugs, as well as mechanisms of drug action.

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