Assessment of heat production, heat loss, and core temperature during nitrous oxide exposure: a new paradigm for studying drug effects and opponent responses

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Kaiyala, Karl J., and Douglas S. Ramsay. Assessment of heat production, heat loss, and core temperature during nitrous oxide exposure: a new paradigm for studying drug effects and opponent responses. Am J Physiol Regul Integr Comp Physiol 288: R692–R701, 2005.—Studies using core temperature (Tc) have contributed greatly to theoretical explanations of drug tolerance and its relationship to key features of addiction, including dependence, withdrawal, and relapse. Many theoretical accounts of tolerance propose that a given drug-induced psychobiological disturbance elicits opponent responses that contribute to tolerance development. This proposal and its theoretical extensions (e.g., conditioning as a mechanism of chronic tolerance) have been inferred from dependent variables, such as Tc, which represent the summation of multiple underlying determinants. Direct measurements of determinants could increase the understanding of opponent processes in tolerance, dependence, and withdrawal. The proximal determinants of Tc are metabolic heat production (HP) and heat loss (HL). We developed a novel system for simultaneously quantifying HP (indirect calorimetry), HL (direct gradient layer calorimetry), and Tc (telemetry) during steady-state administrations of nitrous oxide (N2O), an inhalant with abuse potential, chronic tolerance, withdrawal, and drug dependence, and neuronal regulatory systems in ways that contribute to acute tolerance development to its hypothermia-inducing property. Rats were administered 60% N2O (n = 18) or placebo gas (n = 16) for 5 h after a 2-h placebo baseline exposure. On average, N2O rapidly but transiently lowered HP and increased HL, each by −16% (P < 0.001). On average, rats reestablished and maintained thermal equilibrium (HP = HL) at a hypothermic Tc (−1.6°C). However, some rats entered positive heat balance (HP > HL) after becoming hypothermic such that acute tolerance developed, i.e., Tc rose despite continued drug administration. This work is the first to directly quantify the thermal determinants of Tc during administration of a drug of abuse and establishes a new paradigm for studying opponent processes involved in acute and chronic hypothermic tolerance development.

addiction; drug tolerance; acute tolerance; drug dependence; gradient layer calorimetry; indirect calorimetry

IN AN INFLUENTIAL 1971 REVIEW, Kalant et al. (32) made a convincing case for measuring drug tolerance using outcome variables that are continuously scaled, reliable, dose responsive, and amenable to analysis at the component level. An additional, desirable attribute is mediation by central regulatory mechanisms, reflecting the concept that drug use activates neuronal regulatory systems in ways that contribute to acute tolerance, chronic tolerance, withdrawal, drug dependence, and the potential for relapse in abstinent individuals (25, 35, 48, 49, 51, 55, 63). Core body temperature (Tc) epitomizes each of these attributes and has a long (59) and extensive history as an outcome variable in studies focused on major themes of drug addiction, including mechanisms of drug tolerance (6, 14, 24, 27, 36, 38, 39, 54, 64), mechanisms of drug dependence (62), and, more recently, the genetic basis of initial ethanol sensitivity (8, 9, 12, 46).

An important class of theoretical models contends that effector responses generated by central nervous system (CNS) regulatory systems counter drug effects and thereby contribute to tolerance and that the “growth” of such counter responses over repeated administrations underlies both chronic tolerance development and the pathogenesis of drug dependence and withdrawal (15, 25, 48, 49, 51, 55, 63). This model of tolerance and its putative relationship to withdrawal and dependence can be illustrated in terms of the potent hypothermic effect of ethanol (17, 36) or nitrous oxide (N2O) (30, 52, 54), an abusable inhalant with euphorogenic, anxiolytic, and analgesic properties (16). During an initial steady-state administration of either drug, substantial hypothermia first develops, but subsequently Tc often returns toward baseline or control values, indicative of acute tolerance development (17, 30, 54). Acute tolerance to drug-induced hypothermia is proposed to reflect the reflexive, unconditioned activation of heat-producing/heat-conserving effector responses by negative feedback emanating from the hypothymic state (15, 27, 55). With sufficient repeated administrations, the initial drug dose evokes limited hypothermia (36, 54), indicative of chronic tolerance development. One explanation for this phenomenon is that the animal comes to associate the drug-induced hypothermic state with antecedent environmental and/or interoceptive drug onset cues and now promptly responds to these cues by preemptively activating anti-hypothermic responses (associative tolerance is reviewed in Refs. 55, 63). Withdrawal signs and symptoms are generally opposite in direction to a drug’s primary effects and are proposed to represent tonic or cue-evoked activity of CNS-mediated, drug effect-opposing responses that are revealed when the drug is discontinued (62). Hyperthermia is an example of a withdrawal symptom observed in alcohol-tolerant individuals (18). Drug dependence describes a drug-adapted state in which continued drug use is required to avert withdrawal reactions. Thus drug tolerance, withdrawal, and dependence are all proposed to reflect neuroadaptive responses underlying tolerance development. This view implies that individual differences in the propensity for chronic tolerance development might be positively related to the risk of drug addiction (55). Furthermore, if chronic tolerance development begins with the elicitation of drug-opposing regulatory re-
sponses that contribute to acute tolerance, then the initial magnitude of acute tolerance [a reliable individual characteristic with wide interindividual variability (30)] may be positively related to the rate of chronic tolerance development across repeated drug administrations. This hypothesis, originally proposed by Ramsay and Woods (55), has recent experimental support from our laboratory (53).

Critical experimental evaluations of these conceptualizations regarding the etiologies and relationships among acute and chronic tolerance and drug dependence are strongly warranted given the immense societal and personal costs of drug addiction. Valuable insights will likely flow from experimental strategies focused on the underlying determinants of tolerance development during initial and repeated drug administrations. Because \( T_c \) is determined by the integrated difference between metabolic heat production (HP) and heat loss (HL), we have developed a rodent model to study these underlying determinants during steady-state administrations of a temperature-altering drug of abuse. This experimental paradigm simultaneously records HP via indirect calorimetry, HL via direct gradient layer calorimetry, and \( T_c \) via biotelemetry during exposures to \( \text{N}_2\text{O} \), a drug of abuse with a dose-related hypothermic effect (52) and one uniquely suited to protocols requiring prolonged steady-state administrations and freedom from active metabolites (30, 54, 68). This paper describes our experimental paradigm, reveals in detail the thermal basis of \( \text{N}_2\text{O} \) hypothermia, and sets the stage for future applications to the study of tolerance development and the drug-dependent state.

**MATERIALS AND METHODS**

**Protocols.** Thirty-four male Long-Evans rats (Simonsen) were randomized to receive either \( 60\% \text{N}_2\text{O} \) (n = 18) or placebo gas (n = 16). Mean body mass at test was \( 430 \pm 67 \) (SD) g. Seven to 14 days before test, a battery-operated temperature sensor (MiniMitter VM-FH, Bend, OR) was implanted in each rat’s peritoneal cavity. Rats were maintained on a 12:12-h light-dark cycle on ad libitum pelleted chow diet (lights on at 0700) in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited satellite animal housing room contained within our laboratory. All procedures were approved by the University of Washington’s Institutional Animal Care and Use Committee.

Each rat was placed at \( 1000 \) (±30 min) in the exposure chamber, a \( 19 \times 19 \times 19 \) cm (inside dimensions) Seebeck gradient layer calorimeter (Thermometrics, La Jolla, CA), for measurement of body HL. This device was plumbed and sealed for simultaneous assessment of respiratory gas exchange to enable synchronous indirect measurement of HP. Each exposure session entailed an initial 2-h exposure to a control (placebo) gas approximating room air (21% \( \text{O}_2 \)-79% \( \text{N}_2 \)) blended from medical-grade gases) for abatement of handling-induced increases of \( T_c \) and metabolic activity. All gas blends delivered into the gradient layer calorimeter were regulated via a system of computer-controlled digital mass flow valves with a combined total flow rate of 1.16 l/min STPD, as detailed below. After the 2-h baseline placebo gas exposure, a 5-h test exposure session commenced, consisting of a steady-state exposure to either 60% \( \text{N}_2\text{O} \) (balance 21% \( \text{O}_2 \) and 19% \( \text{N}_2 \)) or continued delivery of the placebo gas. The choice of 60% \( \text{N}_2\text{O} \) stems from our previous work, indicating that it evokes substantial hypothermia and that both acute and chronic tolerance develop to this drug effect (30). To rapidly establish the target concentration of 60% \( \text{N}_2\text{O} \) in the direct calorimeter, the first 5 min consisted of 79% \( \text{N}_2\text{O}-21\% \text{O}_2 \) (flow rate unchanged) before it was returned to the target gas mixture of 60% \( \text{N}_2\text{O} \). This procedure drives \( \text{N}_2\text{O} \) to 60% in <6 min. A custom-built loop antenna raised slightly above the floor of the gradient calorimeter received the \( T_c \) signal, which was transmitted to a receiver by a wire routed through the gas outlet port and then externalized through a gas-tight port. Rats were in a low-walled, square plastic tub (17.5 \( \times \) 17.5 \( \times \) 6.5 cm, Rubbermaid no. 2 Servin’ Saver) for isolation from the antenna components and collection of urine and feces. With tub and antenna in place, the calorimeter volume was ~6.33 liters. Effluent gas samples were measured six times per minute for \( \text{O}_2 \) and \( \text{CO}_2 \) by an indirect calorimeter system, as described below. Effluent gas was analyzed separately for percent \( \text{N}_2\text{O} \) in all studies using an infrared gas analyzer (Normocapoxy, Datex, Helsinki, Finland).

**System components for measurement of body heat flux and storage during drug exposure.** The gas exposure/calorimetry/temperature assessment system is illustrated schematically in Fig. 1. Each gas enters a computer-controlled digital mass flow valve (Sierra Instruments model 840, Monterey, CA) calibrated and tested for accurate, regulated flow of that gas. A custom manifold (Industrial Specialties, Englewood, CO) blends the gas, which then flows through a thermohygrometer (Omega Engineering, model RH411, Stamford, CT) for measurement of temperature and relative humidity of the gas entering the gradient calorimeter. Gas enters the calorimeter through a ceiling port, exits via a port centered on one side wall, and flows through a second thermohygrometer to measure the addition of temperature and relative humidity needed to calculate evaporative HL (EHL) and the (small) component of dry HL (DHL) expressed as increased gas temperature. The gas next flows through a drying column (Drierite 26800, Xenia OH) and then a mass flowmeter (Sierra 822S). Gas samples for measurement of effluent \( \text{O}_2 \) and \( \text{CO}_2 \) fractions are then drawn and delivered via an integrated subsampler pump/mass flowmeter assembly (TR-SS1, Sable Systems, Henderson, NV) to gas analyzers (FC-1B fuel cell \( \text{O}_2 \) analyzer, CA-2A \( \text{CO}_2 \) analyzer, both by Normocapoxy).

Fig. 1. Schematic of model system for studying the determinants of nitrous oxide (\( \text{N}_2\text{O} \)) hypothermia. See MATERIALS AND METHODS for details. A/D, analog to digital.
A computer-controlled baselining unit (TR-BL1, Sable Systems) allows automated selection of effluent or influent gas streams for measurement of O₂ and CO₂ concentration. A drying column on the inlet port of each gas analyzer scrubs remaining moisture.

Performance of the gradient layer calorimeter was checked period-ically with an electric heater at 0.8–3 W. The gas sensors of the indirect calorimeter were calibrated with custom-made, certified 60% N₂O and placebo gas blends (both 21% O₂, Polar Cryogenics, Portland, OR) before each rat was studied.

Air temperature in the lab was 21 ± 1°C (range). The temperature of the gas entering the calorimeter equilibrated with the lab temperature in every exposure session. The six walls of the gradient layer calorimeter house a network of copper tubing (distal to the gradient layer itself) through which constant-temperature water flows (200 ml/min) to create a constant and well-defined thermal surround. The water comes from a tap/regulator assembly and flows through a heat exchanger comprised of 30 m of copper tubing (inside diameter = 0.64 cm) immersed in a 290-liter reservoir of water whose temperature always remained within a fraction of a degree of the mean laboratory temperature.

The initial turnover in the calorimeter’s gas environment after commencement of N₂O flow was studied for the potential to cause artifactual changes in O₂ consumption. Experiments with no rat in the calorimeter established that the effluent gas composition equilibrates with the influent concentration by 10 min after N₂O onset. Indirect calorimetric values were thus excluded for the first 10 min of N₂O exposure to guard against spurious values for O₂ consumption. For equivalence of comparisons, the same data interval was also excluded for placebo gas sessions.

Computer control of system components and data acquisition. A program written in LabVIEW (National Instruments) provided computer control of calorimeter components and data acquisition. Telemetric Tc data were acquired with VitalView software and a Mini-Mitter TR 3000 Receiver and Data Port 24. Mass flow valves were controlled by a multi-channel mass flow controller (MFC-4, Sable Systems). Data from the calorimeter and temperature instruments were delivered to computer files via a multichannel interface (UI-2 Interfacer II, Sable Systems). All calorimeter data were stored at 10-s intervals, whereas data for Tc and the N₂O fraction of the expired gas were stored at 15-s intervals.

Energy computations. DHL was computed by multiplying the gradient calorimeter’s calibration constant (0.16 W/mV) times the millivolt calorimeter signal output. (The fraction of DHL expressed as an increase of the temperature of the gas ventilating the calorimeter was <0.03 W). EHL was computed by multiplying the H₂O mass added to the effluent gas by the latent heat of vaporization (129 J/g). The addition of H₂O was computed from the change of relative humidity between influent and effluent gas, and the Bolton equation (4) was used to calculate saturation vapor pressure. Total HL was computed as the sum of DHL and EHL.

O₂ consumption was calculated based on the difference between influent and effluent O₂ flow rates, as detailed in Ref. 40. The energy equivalent per milliliter of O₂ consumed was computed, assuming a respiratory quotient of 0.85 (the average of the placebo-treated rats), as 1.16 J/ml O₂. N₂O interfered with measurements of CO₂, and we were unable to determine a correction factor due to difficulty in obtaining the requisite certified span gases. Thus we based the energy equivalent of O₂ by assuming a fixed respiratory quotient. This introduces a small source of error (±3%) in estimates of HP.

As one means to evaluate the fidelity of calorimeter estimates of HB, the integral of HB was used to compute a profile of calculated change in Tc, starting at time (t) = 0 for each rat. The rat’s specific heat was taken as 3.47 J/g°C (22). Thus change in Tc (at t = Δt) = ∫₀ᵗ HBdt + l g°C·3.47 J·1·g body mass⁻¹. Total HL was divided by the gradient between Tc and ambient temperature (Tamb), yielding a measure known as “wet” whole body conductance owing to the inclusion of the evaporative HL term in the calculation (21, 22) (Cevap, in W·kg⁻¹·C⁻¹). DHL was divided by the Tc − Tamb gradient, yielding a measure known as dry whole body conductance (Cdab; in W·kg⁻¹·C⁻¹). These alternative measures serve as indexes of the permissiveness with which heat dissipation occurs under different conditions and permit inferences about the peripheral vasomotor state of the animal (21, 22).

Baselining. Baselining intervals during which influent gas is sampled to define and correct for drift in gas sensors were 1.2 min in duration and began at −110 min and then occurred every 20 min during the entirety of each gas-exposure session (21 total baselining intervals). Baselining entailed a 1.2-min diversion of influent gas to sensors (30% of total flow). A mathematical model, assuming that a rat consumes O₂ at a constant rate (8 ml/min, 2.7 W), predicts that the chamber O₂ fraction will decrease during a baselining interval with a time constant of 7.2 min. This decrease results in a transient overes-timate in calculated HP values (6%) when the baselining interval ends. This error then diminishes exponentially with a time constant of 5.1 min, such that the average error is <3% (0.08 W).

Data reduction and statistical analysis. To define temporal profiles of HP, HL, and Tc, data from each rat were averaged over 1-min intervals (means). For groupwise statistical comparisons, each rat’s data were averaged over 20-min intervals. The first 20-min comparison interval after t = 0 (N₂O onset) commenced at 10 min to allow for the initial uncertainty in gas exchange-based energy calculations, as described above. We predicted a priori that N₂O would lower HP and increase HL, based on limited previous work in humans (41, 47). Treatment effects were thus evaluated with between-groups pre-planned t-tests. Additionally, to determine how 60% N₂O influences the relationship between HP and HL among individual rats, linear regression was used to characterize the association between normalized average HP and HL values within each 20-min time bin. In this analysis, each variable for each rat was first averaged within each bin and then transformed into the percent change from the individual’s baseline value. However, although dividing changes of variables by their baseline values as a means of adjusting for the latter has the advantages of easy understandability and widespread acceptance, this method does not truly adjust deltas for differences in baseline. This problem, well recognized by biostatisticians, occurs because both changes and percent changes often are significantly correlated with baseline values simply because all entities have the baseline score in common (42, 43, 58). A statistically rigorous approach to adjusting deltas for baseline is to compute regression residuals from the mean prediction line describing the component of variation accounted for by baseline (43). This estimates the component of the delta that is independent of (i.e., uncorrelated with) baseline. Then, regression residuals can be analyzed for associations. If significant associations remain after such adjustment, confidence is increased that the changes independent of baseline truly are correlated. We employed this meth-o�ogy and report the results in tandem with the simpler baseline-normalized results. Significance was defined as p = 0.05, two tailed.

RESULTS

Average energy fluxes during N₂O/placebo exposure. Inhalation of 60% N₂O initially caused significant reciprocal changes of HL and HP (see Figs. 2, A and B, and 3, A and B). In the first 20-min time bin after commencement of N₂O inhalation, mean HL increased above baseline by 15.8 ± 2.8%, whereas HP decreased by 16.8 ± 3.0%. Together, these changes yielded a pronounced state of negative HB averaging −2.5 ± 0.2 W/kg in first 20-min bin after N₂O commencement. The initial N₂O-induced perturbations of HL, HP, and HB each peaked rapidly and then resolved quickly, such that thermal equilibrium was reestablished at ~120 min (Figs. 2A and 3C); thereafter, HB remained near equilibrium, in agree-
Examination of the partitioning of HL during N₂O administration (Fig. 2F) revealed that DHL peaked at ~15 min and then dropped rapidly to levels that remained persistently below those of the control rats after ~60 min (see Fig. 3D for statistical evaluation). EHL, however, both increased with the onset of N₂O and remained significantly elevated for the exposure duration (Figs. 2F and 3F).

C_{wet} increased sharply during N₂O inhalation and then diminished to values that generally remained significantly above those of the placebo rats (Figs. 2E and 3E). C_{dry} was significantly elevated only in the first two time bins after commencement of N₂O (respective P values of <0.001 and <0.01 compared with placebo controls); thereafter, C_{dry} did not differ between groups.
Individual associations between HL and HP during N₂O exposure. In the earliest time bin during N₂O treatment, baseline-normalized changes of HL and HP were significantly correlated ($P < 0.01$; Fig. 4), suggestive of a coupling between N₂O-induced changes of HP and HL. The data and regression line describing this relationship (Fig. 4A) indicate that N₂O-treated rats with the greatest relative increases of HL generally had the smallest relative decreases of HP. In placebo-treated animals, baseline normalized changes of HL and HP were substantial, directly related, but self-canceling (Fig. 4), as expected given that temperature-stable animals typically show considerable metabolic fluctuation involving parallel changes of HP and HL. Among N₂O-treated rats, percent change in HP in the first 20-min time bin after gas onset was well correlated with baseline HP ($r = -0.71; P = 0.001$), indicating that rats with the highest baseline HP generally evidenced the largest relative drop of HP. Importantly, the associations between change in HP and change in HL each remained significant after adjustment for baseline using linear regression (N₂O: $r = 0.59$, $P = 0.01$; placebo: $r = 0.95$, $P < 0.0001$).

After the first time bin during N₂O administration, the associations between baseline-normalized changes of HL and HP remained significant, and by the third bin, these associations became statistically indistinguishable from those of placebo-treated rats in terms of slope and intercept estimates (Fig. 4C). However, compared with controls, the strength of these associations in N₂O-treated rats generally remained weaker during the first 150 min of gas administration but not during the final 150 min (Fig. 4D). Within all time bins, intragroup associations between changes in HP and HL remained significant after regression-based adjustment for baseline values, and $r^2$ values uniformly matched or exceeded those for the simpler, baseline normalized approach shown in Fig. 4D.

Baseline HP was significantly correlated with change in HP and percent change in HP in all time bins during N₂O exposure, whereas baseline HL was not significantly correlated with...
change in HL and percent change in HL in all time bins. We therefore examined whether baseline HP was a significant predictor of altered HL early during N2O exposure. With baseline HP and change in HP simultaneously included as predictor variables in the regression model, only change in HP was significantly associated with change in HL in the first time bin during N2O exposure (multiple $R^2/H_1005 = 0.59$, $P/H_1005 < 0.02$ for change in HP; $P/H_1005 = 0.21$ for baseline HP). Thus baseline HP was not associated with the predominant avenue of negative HB during N2O treatment.

Individual differences in HP and HL during N2O exposure. Examples of individual differences in profiles of heat flux and $T_c$ among N2O-treated rats are shown in Fig. 5. Shown are data from two rats exhibiting patterns of sensitivity with and without acute tolerance to N2O hypothermia. With baseline HP and change in HP simultaneously included as predictor variables in the regression model, only change in HP was significantly associated with change in HL in the first time bin during N2O exposure (multiple $R = 0.59$, $P = 0.02$ for change in HP; $P = 0.21$ for baseline HP). Thus baseline HP was not associated with the predominant avenue of negative HB during N2O treatment.

DISCUSSION

To our knowledge, this is the first study to simultaneously quantify HP, HL, and $T_c$ during a body-temperature challenge elicited by a drug of abuse. This distinction is perhaps surprising considering the long use of drug-induced $T_c$ changes in studies on drug tolerance and dependence, and because calorimetry/telemetry has been successfully exploited for other aims in humans (60), nonhuman primates (56, 57), and rodents (23, 66). The closest example to our work is that of Gordon and colleagues (23), who used combined calorimetry/telemetry to explore the effects of a neurotensin analog on the determinants of $T_c$.

On average, N2O administration elicited equivalent and unambiguous reciprocal changes of HP and HL, each favoring a loss of body heat (Fig. 2). The temporal dynamics of HP and
HL roughly mirrored one another, reaching maximum excursions in \(<30\) min after \(\text{N}_2\text{O}\) onset, then coursing back toward control levels along curvilinear paths. These profiles fit well with the temporal pattern of \(\text{T}_\text{c}\) observed in the rats, because the mean predicted excursion of \(\text{T}_\text{c}\) derived from individual HP and HL recordings is a reasonable facsimile of the biotelemetrically obtained temperature profile. Furthermore, as illustrated in Fig. 5, the predicted \(\text{T}_\text{c}\) for individual rats was faithful to that rat’s actual \(\text{T}_\text{c}\). This achievement reflects several factors, including the choice of a small-volume gradient calorimeter to shorten response times to changes in HL and considerable pilot work to optimize the protocol for indirect calorimetry, a technology that must be implemented with diligence to minimize potential sources of error (40). We stress the importance of frequent baselining to correct for baseline drift, long a problem bedeviling indirect calorimetry, as well as calibrating gas sensors on the day of each study. It should be noted, however, that the frequency of baselining is predicted to produce a small but transient artifactual overestimation in HP (see MATERIALS AND METHODS), and indeed, this is apparent in profiles of HP (Fig. 2, A and C). The observation that this artifact did not result in a reliable overestimation of HP relative to HL in placebo rats and that the agreement of actual \(\text{T}_\text{c}\) measurements with those based on measurements of HP and HL (Figs. 2, B and D, and 5, B and D) suggests that this error was offset by a small systematic error(s) elsewhere. Although it would be possible to mathematically correct for the baseline artifact or to mitigate it with data smoothing, the most satisfactory approach would be to eliminate it by changing the baselining procedure such that the influent gas flow rate always remains constant.

On average, the balance between HP and HL in the \(\text{N}_2\text{O}\) group did not become sufficiently positive to appreciably drive \(\text{T}_\text{c}\) back toward baseline, i.e., little acute tolerance occurred. It should be noted, however, that individual rats in the present study did exhibit acute tolerance (discussed below). In our previous studies, which used a polycarbonate exposure chamber, the mean \(\text{T}_\text{c}\) profiles during \(\text{N}_2\text{O}\) administration were indicative of significant acute tolerance development (30, 54). Two factors may have worked against the expression of acute tolerance in the present study: 1) the water-cooled metal-walled surround of the direct calorimeter used in the present study likely imposed a greater environmental heat sink than polycarbonate does; and 2) urination resulted in some wetting of the dorsal surface of the rats, which may have worked against heat-conserving physiological responses.

Each of the two measures of whole body conductance, \(C_{\text{wet}}\) (the sum of evaporative and DHL divided by the \(\text{T}_\text{c}-\text{T}_{\text{amb}}\) difference) and \(C_{\text{dry}}\) (DHL divided by the \(\text{T}_\text{c}-\text{T}_{\text{amb}}\) difference) rose sharply after \(\text{N}_2\text{O}\) onset and then returned rapidly toward baseline values. In the \(\text{N}_2\text{O}\) group, \(C_{\text{wet}}\) remained persistently elevated compared with the placebo control group (Figs. 2 and 3), whereas \(C_{\text{dry}}\) became statistically indistinguishable from control after the third time bin after \(\text{N}_2\text{O}\) onset. These findings differ from what would be predicted during forced hypothermia (i.e., from cold stress), which lowers the body’s overall conductance for HL via peripheral vasoconstriction (limiting vascular heat transport to the skin-environment interface) and by inhibiting processes responsible for evaporative water loss (22). Accordingly, the elevation of \(C_{\text{wet}}\) and the absence of a significant decrease in \(C_{\text{dry}}\) in hypothermic \(\text{N}_2\text{O}\)-treated rats can be interpreted to mean that this drug persistently increases whole body conductance by increasing EHL and producing peripheral vasodilation. Note that the present methodology carries a limitation regarding the interpretation of the \(\text{N}_2\text{O}\) group’s increase of EHL because steps were not taken to
prevent urinary evaporation. Accordingly, the increase of EHL during N2O inhalation might reflect increased urinary output as opposed to increased respiratory water loss and saliva spreading, as might be predicted if N2O reduced the set point for Tc (discussed below). In studies specifically designed to address this issue, urine must be isolated from the rat as described by Gordon and colleagues (23).

Existing data suggest that N2O inhalation elevates peripheral (skin) blood flow resulting in a redistribution of body heat from core to shell (45). Administration of 30–35% N2O in humans reliably increases indirect estimates of skin blood flow (Ref. 47 and unpublished results from our laboratory). This increase could stem from a withdrawal of tonic thermoregulatory vasomotor constriction, as proposed for the volatile anesthetics (45). Ethanol-induced hypothermia also involves increased peripheral blood flow that is mediated via central vasomotor control mechanisms (37). The neurochemical mediators through which inhaled N2O might increase peripheral blood flow could involve nitric oxide (NO), a potent vasodilator with an important role in blood flow distribution (69). A role for NO in N2O-augmented heat dissipation seems plausible in part because Quock and colleagues (7, 26, 28) have implicated NO in other N2O effects, specifically its antinociceptive and anxiolytic effects. Additionally, because hypoxia-induced hypothermia is attenuated by microinjection of a NO synthesis inhibitor in the preoptic hypothalamus (65), it is possible that central as well as peripheral NO release could participate in mediating the hypothermic effect of N2O.

The mechanism of HP suppression by N2O is unclear but could reflect its sedative effect resulting from its action as a NMDA receptor antagonist (29). Alternatively, N2O could act more specifically at sites involved in the neuronal control of metabolic rate. On the basis of in vitro work, N2O does not appear to be a thermogenesis inhibitor in rodent brown adipocytes (44), a major organ of facultative thermogenesis in rodents. Thus N2O suppression of HP probably does not reflect a direct effect on brown adipocytes. Some researchers propose that the hypothermia evoked by administration of certain drugs (notably ethanol), and by hypoxia, reflects an adaptive lowering of the CNS thermoregulatory set point as a biological strategy to reduce cell damage and sensitivity to toxic drug effects (11, 13, 20, 23). Limited research places N2O within this context in that N2O-treated mice have been reported to actively seek a cooler ambient temperature in a temperature gradient (50). The coordinate reciprocal changes of HP and total HL concomitant with the increases of whole body conductance and EHL observed in our study are consistent with the possibility that N2O elicits a regulated hypothermia, as proposed for ethanol (11, 13, 31). This perspective thus views the hypothermic state induced by N2O or ethanol as a change that the body seeks rather than repels. By contrast, various contemporary models of drug tolerance development (25, 48, 49, 51, 55) regard the drug-induced hypothermia as a state that the body opposes, consistent with the observation that rats clearly develop both acute and chronic tolerance to hypothermia induced by ethanol or N2O (17, 30, 31, 53, 54). An interesting goal for future research would be to reconcile these seemingly disparate perspectives. This issue may have important ramifications for better understanding the stimulus state that elicits the adaptive responses to drug administrations, as well as for better understanding the role of hypothermia in protection from toxicological insults. Studies to elucidate the impact of acute and chronic N2O administrations on physiological and behavioral thermoeffector responses (including measures of skin blood flow and choice of location in a temperature gradient) could cast needed light on this issue.

Relative changes of HL and HP in N2O-treated rats were strongly associated even after statistical adjustment for baseline values, such that rats with the greatest change of one had the smallest change of the other (Fig. 4). This association points to individual differences in the predominant physiological mode of the hypothermic effect of N2O, i.e., lowered HP vs. increased HL. The concept that animals differ in the recruitment of specific mechanisms during homeostatic perturbations dates to at least 1953, when Kanter reported that some dogs corrected body-fluid imbalances primarily by drinking, whereas “others used their kidneys” (p. 93) (33).

Individual profiles of hypothermia in rats during prolonged steady-state N2O administrations can differ widely in terms of initial sensitivity and acute tolerance development (30, 54). The data shown in Fig. 5 illustrate this phenomenon and speak to its origins. One rat (Fig. 5, A and B) developed considerable hypothermia and did not recover from this state, consistent with the average pattern evidenced by the group overall. A second rat (Fig. 5, C and D) illustrates acute tolerance, i.e., after becoming hypothermic, Tc returned toward baseline owing to the development of a persistent state of positive HB. Switching to a positive HB is consistent with the concept that drug-induced hypothermia serves as a stimulus that elicits warming responses. From the standpoint of formal control theory, the deviation of Tc from its set point constitutes an error signal that acts in the CNS to elicit corrective alterations in HL and HP. Because HL and HP act on the same summating point (Tc), fairly subtle individual differences in the regulatory parameters according to which of these mechanisms respond [control system operating characteristics such as threshold, gain, phase lag (3, 15)] could produce substantial differences in patterns of acute tolerance during N2O administration. The existence of more extreme individual differences in regulatory parameters is also suggested by other research (30).

First, although each of the rats given N2O in the present study were sensitive to the drug’s hypothermic effect, previous research from our laboratory has documented considerable individual variability in this outcome. In fact, some rats appear insensitive to the temperature-lowering effect of N2O in an initial administration (the least sensitive 10% exhibit <0.5°C of maximal hypothermia to 60% N2O) (30). Second, in ongoing research using the paradigm described in the present paper, rats have been identified that appeared initially insensitive to N2O at the level of Tc, but were clearly sensitive to the drug’s initial HL-increasing effect. For example, one N2O-naïve rat that exhibited little hypothermia (maximum decrease of 0.5°C) did so despite a 28% mean increase of HL in the first 20-min bin after N2O onset compared with the 20-min interval before drug onset. This rat’s limited hypothermia development resulted from a prompt increase of HP that largely negated the increase of HL. Thus our methodology has considerable potential for investigating the etiology of individual differences in initial drug sensitivity as well as acute tolerance development.
Perspectives

Over 30 yr ago, Kalant and colleagues (32) recognized that the phenomenon of acute (intrasessional) tolerance might be mechanistically linked to chronic tolerance development, stating that the “fundamental distinction to be made, then, is between intrasessional adaptation (acute tolerance) and intersessional adaptation (chronic tolerance). The empirical and mechanistic relationship between them must be investigated in greater depth” (p. 147–148). The approach described in this paper has unique potential for addressing this question. Additionally, our system can obviously be extended to study tolerance in rodent models involving a wide variety of abused drugs known to alter Tc, including the active ingredient of marijuana (67), morphine (hyperthermia) (1, 2, 19), cocaine (hyperthermia) (10), methamphetamine (5), nicotine (hyperthermia) (61), heroin (34), and ethanol (6, 8, 9, 12, 14, 17, 24, 27, 36, 38, 39, 46, 64). Ethanol represents a particularly attractive candidate for future studies because its blood concentration can be maintained at near steady-state levels (17), which facilitates studying acute and chronic tolerance. We are particularly interested in applying our paradigm to studying the associative basis of tolerance development to determine the extent to which interoceptive and/or environmental cues can elicit conditioned compensatory responses that are evident at the level of HP and HL.

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