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Review

Thermoregulation and its influence on toxicity assessment☆

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Abstract

The thermoregulatory system of laboratory rodents is susceptible to a variety of chemical toxicants. Because temperature directly affects the reaction of virtually all biological processes, it is critical to consider how changes in the thermoregulatory response to a toxicant may affect physiological, behavioral, and pathological endpoints. Researchers in industry and government laboratories are often faced with addressing how changes in body temperature of their experimental subjects may affect the outcome of a particular toxicity test and/or screening panel. However, many toxicologists are either unaware of the importance or ignore the potential impact of a toxic-induced change in body temperature. This paper endeavors to summarize the importance of thermoregulation in the study of toxicology and propose recommendations for thermometry that researchers may utilize in their toxicological studies.

Keywords: Radiotelemetry; Core temperature; Skin temperature; Pesticide; Rodent; Metabolic rate; Inhalation; Stress; Restraint

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1. Introduction

The Society of Toxicology sponsored a symposium in 2006 entitled, "Thermoregulation and its influence on toxicity assessment". The speakers in the symposium posed compelling arguments for making toxicologists more aware of the importance of thermoregulation in toxicology assessment. First, while body temperature is normally stable, altered regulation is seen upon exposure to toxicants, with the effects usually being more pronounced in smaller mammals. Second, while large mammals such as humans may regulate a stable core temperature, environmental heat and cold stress will nonetheless exacerbate the physiological and behavioral responses to a toxicant. Third, by virtue of the stable nature of the core temperature, any toxicological insult that changes temperature should be considered as a biologically significant event. Hence, one should consider the biological importance when a toxicant affects body temperature because such an event implies a significant change in physiological homeostasis.

In this paper we strive to show the importance of temperature regulation in the study of toxicology. This is a critical area because toxicity tests, screening panels, and other approaches used in industry and government research laboratories are often faced with addressing changes in body temperature of their experimental subjects. This paper will also show that many researchers are in need of specific recommendations to best characterize the thermoregulatory responses of their rodent test subjects when exposed to toxicants or drugs. To this end, we have developed a table of recommendations for selection of appropriate thermometry methods for toxicology and pharmacology studies.

2. Fundamentals of thermoregulation

The thermoregulatory system of mammals and birds has evolved to regulate a stable internal body (i.e., core) temperature over a relatively wide range of exposure to environmental heat and cold stress, exercise, and fever. Achieving a controlled body temperature can be explained with a simplified version of the heat balance equation (IUPS, 2001) where the change in heat storage of the body (S) is equal to the difference between the body's heat production (HP) and total avenues of heat loss (HL) through convection, conduction, radiation, and evaporation:

$$\Delta S = \mathrm{HP} - \mathrm{HL} \quad (1)$$

When ΔS is equal to zero, then core temperature is stable. If the sum of metabolic heat production from basal metabolism and exercise exceeds the total avenues of heat loss, then ΔS is positive and hyperthermia will develop. Conversely, if heat loss exceeds the heat production capacity then the animal will become hypothermic. A major goal of thermal physiology is to understand the physiological and behavioral mechanisms involved in the regulation of thermoeffectors for control of heat production and heat loss in homeothermic organisms. In these species a change in body temperature is often considered to be the result of an acute dysfunction of the homeostatic processes controlling temperature. The change in body temperature is a useful, integrative tool in toxicology; a temperature change can be an alert for possible rodent-specific toxicological effects, despite the yet unknown cause of the thermoregulatory response. Hence, changes in body temperature can be taken as early sensitive, albeit a non-specific indicator of changes in behavioral and autonomic thermoeffector function.

3. Applying thermoregulation to toxicology research

Since all biochemical and physiological processes are directly affected by temperature, toxicologists should verify that a particular endpoint affected by a toxicant is not actually an indirect result of the toxicant changing body temperature. The principle of the Arrhenius equation is based on thermal kinetics and states that the rate of chemical reactions increases exponentially with a rise in temperature (Schmidt-Nielsen, 1975). Thermal biologists often use the Q_{10} to express the effects of temperature on enzymatic reactions, physiological processes, etc. (Fig. 1). In general, biological processes increase by two- to three-fold for each 10 °C increase in temperature. Physiological processes such as nerve conduction velocity, axonal transport, heart rate, cell division, and tissue metabolism have positive temperature coefficients, meaning they increase in activity with

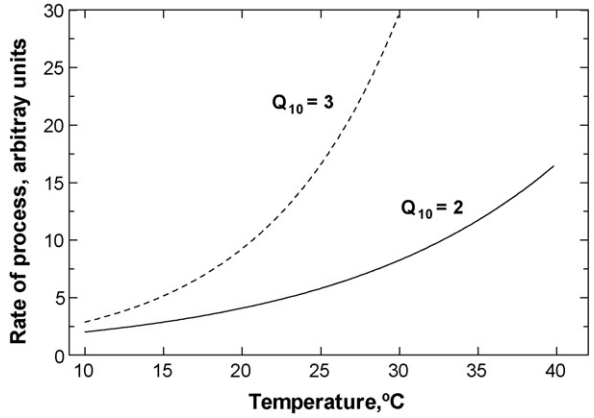

Fig. 1. A diagrammatic representation of the effect of temperature on the rate of a chemical reaction or physiological process. Theoretical functions show the effects of a doubling ($Q_{10} = 2$) or tripling ($Q_{10} = 3$) the rate of the process with a 10 °C increase in temperature. Adopted from Schmidt-Nielsen (1975).

a rise in temperature. On the other hand, electrical resistance of excitable membranes and the height of neuron action potentials increase in magnitude with cooling (for review, see Gordon, 2005).

Some toxicologists are aware that the toxicant they are testing has some type of effect on the thermoregulatory system. They may note that their test subjects are exhibiting behaviors that suggest a change in thermoregulation such as the animals huddling together or burrowing into the bedding material after dosing, suggesting that the animals are hypothermic; or the animals may show a splayed behavior, suggesting they are hyperthermic. Some investigators may report that the animal's skin feels hot or cold after dosing and may infer (incorrectly) that the animals are hyperthermic or hypothermic. All together, this paper endeavors to educate and promote awareness of thermoregulatory responses of rodents and other species in toxicological investigations.

4. Thermometry: advantages and pitfalls

From the aforementioned discussion, it is clear that toxicologists should consider how to better monitor body temperature and possibly other thermoregulatory endpoints of their test subjects. Accurate measurement of a seemingly simply parameter is often a challenge to the toxicologists. Using a colonic or rectal probe to measure the core body temperature is in fact an accurate measurement of the core body temperature; however, the handling of the animal and insertion of the probe is stressful to mice and rats and their body temperature will rise or fall for several hours depending on the species and degree of handling (for review, see Gordon, 1990, 1993). The first time measurement of the resting rat or mouse with a fast-responding thermocouple or thermistor probe will provide an accurate estimate of the resting core temperature. However, repeated insertion of the probe at regular intervals (e.g., $\leq$1 h) will yield artifactual measures of the true body temperature because the stress of the repeated measurements leads to prolonged hyperthermia in rats and mice. Not surprisingly, restraining the animal and insertion of the temperature probe also leads to stress and aberrant thermal responses (for more discussion, see Section 7).

Overall, in the application of thermometry to measure core temperature of rodents, one should assume that the accuracy of the measurement is compromised with any disturbance, handling, or even the presence of personnel near the animal. To this end, we have developed a framework of recommendations for consideration by researchers in academic, government, and industrial settings (Table 1). We have attempted to prioritize the most desirable methods for thermoregulatory assessments in rodent drug and chemical toxicity studies. Radiotelemetry provides researchers with an ideal means of monitoring core temperature in undisturbed animals continuously for hours, days, or weeks depending on the needs of the experimenter. Providing the animals with an environment that is near thermoneutral conditions is considered ideal. This is achieved by manipulations in the ambient temperature or providing adequate bedding for behavioral thermoregulation. One drawback is that the telemetry devices have to be implanted under surgical anesthesia. Microchip transponders that can encode animal identification number and temperature can be implanted under the skin in unanesthetized rodents (Biomedic Data Systems; www.bmds.com). However, these transponders provide a measure of the subcutaneous temperature, which is influenced by the skin and core temperature. This may or may not provide information needed to judge the true thermoregulatory state of the animal and to assess the thermoregulatory effects of toxicant exposure. The suitability of the transponders as a measurement of the core temperature will vary depending upon the species (Hartinger et al., 2003).

Skin temperature may give clues to the thermoregulatory state of the animal but it is an incomplete picture. For example, if a mouse or rat feels cold (especially the tail) to the touch or by measurement with a surface temperature probe, it may mean that there is peripheral vasoconstriction of blood flow to the skin but the animal may not necessarily be hypothermic. In fact, during the initial stages of a fever, the principal thermoregulatory response is restriction of blood flow from the core to the skin to reduce heat loss and raise core body temper-

Table 1
Recommendations for rodent thermometry in toxicology studies, starting with the most ideal and ending with least ideal method

1. *Radiotelemetry*. Monitoring of a temperature probe surgically implanted in a thermal core of the animal (abdomen, brain). Remote monitoring of animals housed in a vivarium isolated from disturbances from personnel. Maintenance of animals in a thermoneutral environment and/or with bedding that allows animals to adjust insulative qualities of microenvironment
   Disadvantages: expensive start up costs; surgical implantation with anesthesia required
   Advantages: provides best measure of true thermoregulatory patterns of mice and rats; continuous, automated monitoring of core temperature 24 h/day. Allows for observations of acute, sub-chronic, and chronic effects of drugs and toxicants
2. *Subcutaneous transponder ID chip*. ID chips implanted under skin with trocar
   Advantages: less costly than telemetry; accurate measure of the subcutaneous temperature; no surgery or anesthesia required.
   Disadvantages: subcutaneous temperature is not a measure of the core temperature; personnel must be in close proximity to animal to position receiver to collect temperature data. Data collection not automated; no 24 h monitoring
3. *Infrared thermography*
   Advantages: provides non-contact measurement of skin temperature which can be valuable for interpreting effects of toxicants and drugs. Hand held devices relatively inexpensive
   Disadvantages: skin temperature does not provide any indication of core temperature; continuous, automated monitoring is costly. Personnel must be in close proximity to animal
4. *Colonic/rectal temperature probes* (*unrestrained rodents*). Used on single housed animals undisturbed immediately prior to measurement
   Advantages: inexpensive and easy to perform; provides an accurate, a single time point measurement of the core temperature provided animal is undisturbed prior to probe insertion
   Disadvantages: technique evokes stress resulting in change in body temperature; repeated use of device leads to inaccurate estimates of the core temperature due to stress. Difficult to use with multiple housed animals because disturbance causes stress and elevation in temperature as multiple animals are being measured
5. *Colonic/rectal temperature probes in semi-restrained animals* (*e.g., probe inserted and taped to tail*)
   Advantages: inexpensive, accurate measure of core temperature
   Disadvantages: continuous stress of probe insertion leads to abnormally high body temperatures in rats. Animals must be restrained or carefully observed to assure probe remains in place
6. *Restraint with colonic probe*
   Advantages: restrained animal easy to manipulate (i.e., nose-only inhalation)
   Disadvantages: marked effects on thermoregulatory system. Rodents are much more sensitive to ambient temperature when restrained. Unable to use behavior and thermoeffectors as effectively when restrained

The above is a list of recommended methods along with their advantages and disadvantages.

ature. Likewise, peripheral vasodilation increases skin temperature, a response that may be a prelude to lowering body temperature. Measuring skin temperature with non-invasive techniques such as infrared thermography does give the researcher an indication of the possible thermoregulatory responses of their test subject. However, the skin temperature measurement along with core temperature measurement gives a more complete picture of the integrated thermoregulatory response to the toxicant. Insertion of a colonic probe in a restrained rodent is considered one of the least desirable methods of measuring the core temperature (Table 1).

4.1. Neonates

Researchers working with pre-weanling rats and mice are cognizant of the thermal lability of this life stage. Rats, mice, hamsters, and gerbils below the age of 2 weeks posses little homeostatic control of body temperature (for review, see Gordon, 1990, 1993). Prior to maturation of their autonomic motor responses to regulate heat gain and heat loss, these species must rely on behavioral thermoregulation, utilizing the heat produced from the dam and insulation provided by huddling with littermates to achieve a regulated core temperature. Measuring the temperature of these immature animals can be a challenge. Routine handling and dosing that would lead to transient elevations in core temperature of adult rodents may induce marked hypothermic or hyperthermic responses in immature rodents. That is, simply picking up a neonate and holding it in a gloved hand for a routine procedure will be associated with dramatic cooling of the body core unless precautions are taken to prevent the heat loss (Pauluhn and Schmuck, 2003). In view of the fragile nature of their developing tissues and their sensitivity to ambient temperature when removed from the dam and litter mates, application of rectal/colonic temperature probes in neonatal rodents should be carried out with extreme care. Radiotelemetry has been utilized to study the toxicological responses of rat pups as young as 17 days (Mack and Gordon, 2007). The development of miniature, battery-less units

(e.g., Minimitter Corporation, Bend, OR, USA) should allow for application of telemetry in rat and mouse pups at much younger stages of development. Infrared thermography can also be a useful tool to study the thermal state of immature rodents.

4.2. *Effects of restraint on body temperature in rodent inhalation studies: indicator of adverse effect or rodent-specific adaptive response?*

Inhalation studies with rodents are often designed with nose-only exposure which means the animal has to be restrained for long periods of time while being exposed These restraint procedures lead to marked thermoregulatory changes in the test subjects. Restrained rodents are unable to use postural adjustments to behaviorally thermoregulate and cannot apply saliva to their fur to increase evaporative water loss. The stress from restraint also elicits a sympathetic response resulting in vasoconstriction. Rats usually develop hyperthermia whereas mice often become hypothermic when restrained at standard room temperatures (Gordon, 1993; Van Eijl et al., 2006). There is surprisingly little known about the mechanism of action for the opposite core temperature responses of restrained mice and rats. Repeated training in restraint devices can alleviate some of the thermoregulatory deficits. One study estimated that full adaptation, as based on minimal elevations in core temperature and heart rate, required 14 days of repeated placement in a restrainer (Narciso et al., 2003). Regardless of adaptation, it should be clear that the restrained rat or mouse will most likely have altered thermoregulatory responses compared to the free-moving, undisturbed animal.

In rodent inhalation studies it is important to recognize potential biases in toxic outcome that may be caused or modulated by changes in body temperature. Studies using rectal temperature probes along with subcutaneous chip transponders illustrate the utility and limitations of these thermometry techniques with restrained rodents (Pauluhn and Mohr, 1999, 2000). Not surprisingly, the measurement with the transponder chip shows a greater drop in temperature in mice than in rats under similar methods of restraint (Fig. 2). The comparison of findings from mice and rats with subcutaneously implanted transponders revealed a stable body temperature after nose-only exposure of about 30 min, without any appreciable time-related change over the remaining exposure period of 5.5 h. Following repeated restraint sessions, rats did not show differences in body temperatures due to adaptation, while in mice the hypothermic response after the first period of restraint was markedly reduced

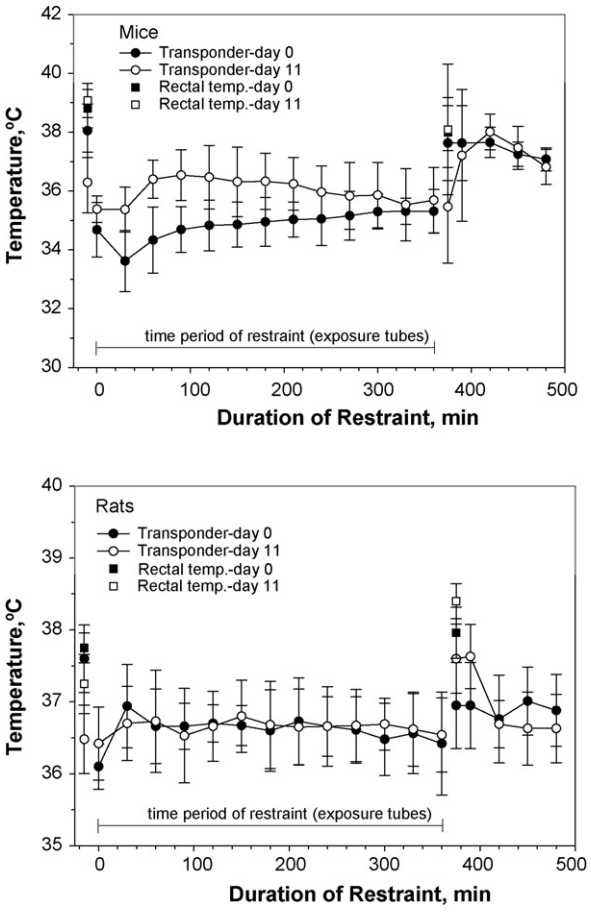

Fig. 2. Effect of restraint on the rectal temperature or subcutaneous temperature of male mice and rats during repeated 6 h nose-only exposures (sequence of exposure days: 0, 2, 3, 7, and 11). Data shown before and after the restraint period were collected in the animals' home cages (single housing). Measurements were repetitively performed with subcutaneously implanted transmitters or by rectal probes on the days shown. Data represent means ± S.D. ($n = 5$). Data from Pauluhn and Mohr (1999).

with training in the restraint tube. In both species, the handling of animals after cessation of the restraint period caused a sudden rise in temperature (mice: ~3 °C, rats: ~1.5 °C) independent of the location of probe (subcutaneous or rectal). These findings suggest that the timing of measurement of body temperature relative to dosing (exposure) with the inhaled toxicant is critical.

Overall, the thermoregulatory response of rodents is essential in the design and materials used in nose-only restraint systems. Thermoregulatory stability will be easier to achieve in nose-only exposure tubes allowing for convective heat exchange from the tail and other surfaces critical for heat exchange. But this may be a benefit or detriment depending on the species, ambient temperature, humidity, and air movement. Restraining devices

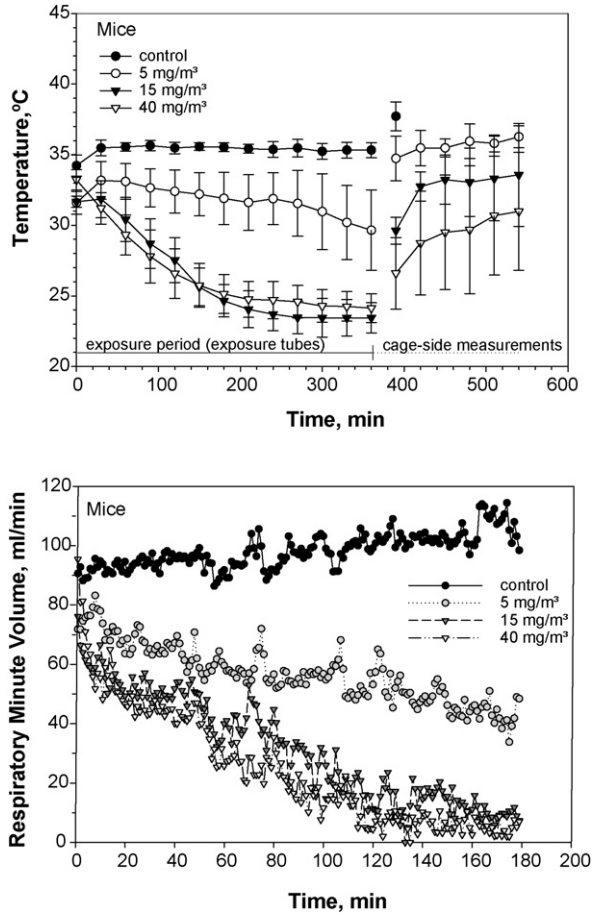

Fig. 3. Relationship of a singe 6 h nose-only inhalation exposure of male mice to an upper respiratory tract isocyanate on the body temperature (upper panel) and respiratory minute volume (lower panel). Body temperatures were measured by subcutaneously implanted transmitters. After cessation of exposure, measurements were made in the animals' home cage (individual housing). Respiratory measurements utilized volume displacement plethysmographs during the first 3 h of exposure ($n = 8$). Data from Pauluhn (2004).

made of plastic will impede heat loss at warm temperatures, potentially exacerbating a hyperthermic state but attenuating a hypothermic response in the cold. Devices made with thermally conductive metals will facilitate heat loss at warm temperatures, but accelerate heat loss, promoting a hypothermic response. Therefore, in the reporting of results from rodent inhalation studies, the characteristics of the restraining device, as it relates to how it may facilitate or impede heat exchange, is a critical parameter to report (Pauluhn and Mohr, 1999, 2000).

It has been shown that acute inhalation exposure to test substances which cause upper respiratory tract sensory irritation may trigger a substantial hypothermic response (Fig. 3). In this example, inhaled doses of isocyanate lead to profound hypothermia and bradypnea in restrained mice, with body temperature approaching 23 °C. Recovery started with cessation of exposure and was dependent on the extent of hypothermia. In rats acutely exposed to a variety of substances and mixtures using the OECD 403 protocol (OECD, 1981) the magnitude of hypothermia after exposure was predictive for the severity and duration of respiratory distress-related clinical signs. Core temperatures as low as 26 °C are seen in rats maintained under severe exposure conditions. The irritant-induced stimulation of sensory nerve endings located in the upper respiratory tract is considered to be a mechanism responsible for the changes in respiration. The triggering of concentration-dependent reduction in respiratory minute volume due to reflexively induced bradypnea clearly coincides with a hypothermic response. This relationship was established with different irritant substances (Pauluhn and Machemer, 1998; Pauluhn and Mohr, 1999, 2000; Pauluhn, 2003). In contrast, substantial changes in body temperature were not observed following acute exposure of rats to lower respiratory tract irritants (Pauluhn, 2004).

5. Toxicant-induced hypothermia

Hypothermia is often seen in rats and mice exposed acutely to toxicants, such as insecticides, heavy metals, solvents, and air borne agents (Gordon, 2005). Many older studies suggested that toxicant-induced hypothermia reflected the onset of a poikilothermic state in which core temperature passively tends to move towards the ambient temperature. This interpretation implies that the toxicant impairs thermoregulatory function, because poikilothermia in homeotherms stems from impairment of the thermoregulatory effectors that preserve thermal homeostasis in the test environment. It is important to note that the majority of whole animal toxicology studies have been performed at standard room temperature (21–24 °C, which is approximately 6–9 °C below the thermoneutral zone for mice and rats (Gordon, 1993 for review). This means that rodents tested in the typical laboratory environment will become hypothermic if a toxicant causes a significant impairment in heat production.

However, the assumption of thermoregulatory dysfunction was questioned in the 1980s when researchers began showing that when rats or mice allowed to behaviorally thermoregulate in a temperature gradient preferred colder temperatures when faced with acute toxic exposure (Gordon et al., 1988; Gordon, 2005). Acute exposure to various insecticides, heavy metals, and solvents elicited a movement to the cool end of a temperature gradient and this behavioral response could

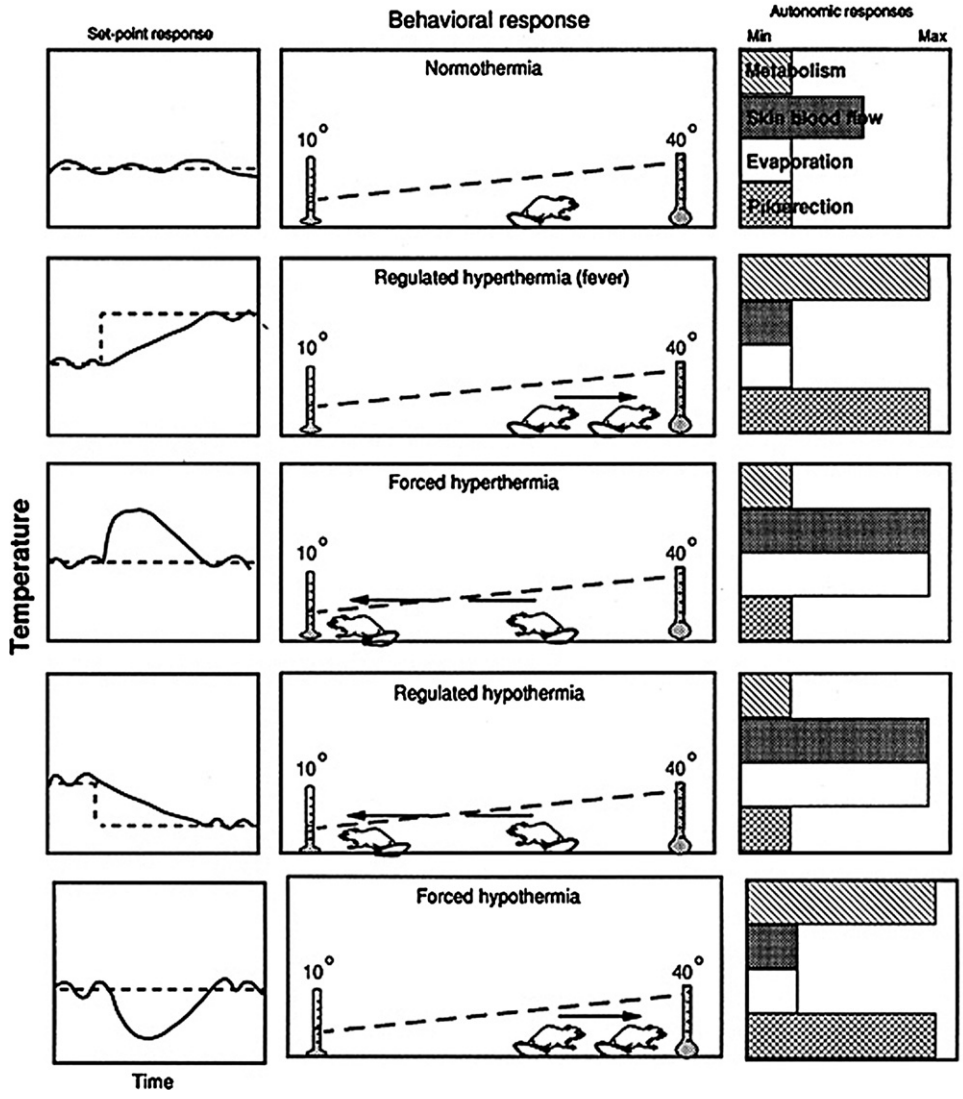

Fig. 4. Summary of behavioral and autonomic responses of a homeotherm when subjected to manipulation of body and set-point temperature: normothermia, regulated hyperthermia (fever), forced hyperthermia, forced hypothermia, regulated hypothermia. Modified from Gordon et al. (1988).

be shown to precede the reduction in body temperature. Generally, the behavioral preference for cooler temperatures coincides with a reduction in heat production and an increase in tail skin temperature, indicating peripheral vasodilation. Thus, the behavioral and autonomic response to reduce heat production and increase heat loss indicates that the toxicant is directly affecting the CNS and eliciting a decrease in the set-point for body temperature (Fig. 4). This is essentially opposite to that of a fever where the thermoregulatory system responds to an infection by reducing heat loss (peripheral vasoconstriction), increasing heat production, along with a preference for warmer temperatures with a resultant elevated core temperature. With toxicant-induced hypothermia, the animals eventually prefer warmer temperatures in the gradient as part of the recovery from the hypothermia. In toxicology, it is rare to find instances where rodents subjected to an acute toxic insult and allowed to behaviorally thermoregulate will select warmer temperatures to prevent the toxic-induced hypothermia (Gordon et al., 1988; Watkinson and Gordon, 1993; Gordon, 2005).

5.1. Benefits of hypothermia

It is well known that the acute effects of most toxic agents are ameliorated with a mild reduction in

body temperature (Gordon, 2005). This would suggest that the regulated hypothermic response to toxic insult is adaptive, leading to increased chances of survival. Lowering body temperature can reduce the toxicity of many chemicals because all the biochemical reactions that may lead to cellular damage, such a formation of reactive oxygen species and lipid peroxidation, are temperature dependent. A lower temperature also slows the metabolic activation of certain classes of toxicants, meaning the production of a toxic metabolite would be slowed with hypothermia. On the other hand, lower temperatures also reduce the clearance and excretion of toxicants. In other words, hypothermia can both protect and exacerbate chemical toxicity. Doull (1972) provided a succinct description in a review of the effects of temperature and other physical factors on toxic response, "Temperature is directly correlated with the magnitude and inversely correlated with duration of drug response in biological systems". Overall, the protective effect of a reduced temperature on cellular toxicity appears to outweigh the undesirable effect of slower clearance of the toxicant from the body.

In many toxicological and pharmacological studies, the hypothermic effect of a chemical is blocked by raising ambient temperature or by placing the animal on a heating pad. Based on the foregoing discussion, such attempts to maintain thermal homeostasis are most likely exacerbating the toxicity of the test agent. Because the rodent exposed to the toxicant would normally activate heat loss mechanisms, exposing the animal to a warm environment may place additional stress on heat dissipating mechanisms. Thus, in the "thermally clamped" test subject, the outcome of the study may be compromised because (i) the cellular toxicity of the chemical agent is exacerbated because of warmer body temperature and (ii) the additional heat stress resulting from a regulated hypothermia in a warm environment places stress on other physiological systems.

6. Thermoregulation: an endpoint to assess toxic sensitivity and tolerance

Core temperature has been utilized by ethanol researchers for decades to define ethanol efficacy during acute and chronic administrations (Palmer et al., 2002; Lovinger and Crabbe, 2005). However, using thermoregulation as an endpoint or bench mark in toxicity studies can entail much more than simply measuring a change in core temperature. The activation or suppression in thermoeffector function can occur at levels of toxicant exposure that cause little or no change in body temperature. Understanding these responses can provide a better characterization of the dose–response of the toxic agent. Thermoregulatory parameters other than core temperature have often been utilized by ethanol researchers to define acute and chronic endpoints of drug efficacy (Palmer et al., 2002; Lovinger and Crabbe, 2005). Measuring the dynamics of heat production, heat loss, and core temperature can be an ideal systems-level approach to study drug and toxic chemical sensitivity. Studies by Kaiyala and Ramsay on the thermoregulatory response of the rat to nitrous oxide (N_2O) have brought attention to this area of study. N_2O elicits hypothermia in the adult rat but only at concentrations of $\geq$60% (Fig. 5; Kaiyala et al., 2007a). However, by measuring the heat loss and heat production of the rat while housed in a direct calorimeter, they were able to show that lower percentages of N_2O elicited a marked rise in heat loss, but there was no change in core temperature because rats would compensate for the increase in heat loss with an equivalent elevation in heat production (Fig. 5). The balanced response of heat production with heat loss resulted in a stable core temperature. The HL response at sub-threshold doses for affecting core temperature suggests that HL is a reliable indicator for a primary drug effect. With repeated chronic exposure to N_2O in adolescent rats, the HL response appears to be countered with an HP response resulting in N_2O-induced hyper-

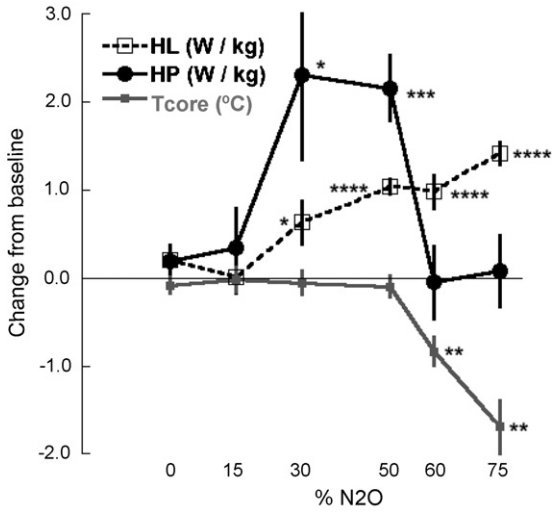

Fig. 5. Effect of inhaled nitrous oxide exposure on heat loss, heat production, and core temperature of rats housed in a direct calorimeter. Note that while core temperature homeostasis was maintained during both 30% and 50% nitrous oxide administration, this stability occurred despite increases in heat loss because compensatory increases in heat production prevented a net change of body heat content. Modified from Kaiyala et al. (2007b).

thermia (Kaiyala et al., 2007b). The relative robustness of the heat production and heat loss changes during toxicant administration could, as is shown with N_2O, explain the inter-individual variability in sensitivity to a toxicant.

7. Extrapolating thermoregulatory responses from rodent to human

Nearly all researchers studying rodents have an interest in extrapolating the significance of their findings in terms of the potential responses of humans. There are, of course, many biochemical, physiological, and behavioral differences between humans and rodents. At first glance, one might think that thermoregulatory differences between species would not be an issue worthy of great concern because the laboratory rat and adult human have nearly identical core temperatures of 37 °C (we should point out that the rabbit and guinea have body temperature at least 1 °C higher than that of the rat and human). The similarity in body temperature between humans and rats is remarkable but can be misconstrued because each species achieves a regulated core temperature through different mechanisms. For example, the metabolic rate of a 300 g rat normalized to body weight is approximately five times higher than that of an 80,000 g human. For a 30 g mouse, the metabolic rate is approximately 10-fold greater than a human. As size decreases, surface area:volume increases. Hence, for homeotherms (birds and mammals), metabolic rate increases with decreasing size in order for heat production (normalized to body weight) to match heat loss (Schmidt-Nielsen, 1975).

The ease with which heat can be dissipated to the environment can be expressed in terms of whole-body thermal conductance (C):

$$C = \frac{M}{T_c - T_a} \quad (2)$$

where metabolic rate (M) in dimensions of W/kg, core temperature (T_c) is measured in at ambient temperatures (T_a) below the thermoneutral zone, and thermal conductance (C) is expressed in dimensions of W/kg/°C. The term C can be viewed as a single coefficient that is a summary of the complex ways in which heat can enter or leave the body through convection, radiation, conduction, and evaporation.

Researchers studying mammals with body weights below 10 kg have shown an allometric relationship between body weight and thermal conductance. An inverse relationship between these parameters illustrates how small size means a greater rate of heat loss at a given ambient temperature (see Gordon, 2005). For example, the thermal conductance of a 30 g mouse is approximately 3.4-fold greater than that of a 300 g rat and 10-fold greater than a 3000 g rabbit. This relationship essentially shows the impact of body mass on the potential for how fast a homeotherm can lower its body temperature. Small mammals cool faster which also means they have greater metabolic cost to thermoregulate when compared to larger species when all other environmental factors are equal. This principle should have a bearing on how a toxicant affects body temperature, especially when the mechanism of action of the toxicant is an inhibition in metabolic thermogenesis.

Body temperature and thermoregulation can also influence the interpretation of pharmacokinetic data. Temperature variation, whether chemically or environmentally induced, can affect the absorption, distribution, metabolism and elimination of xenobiotics. Physiological changes, such as ventilation and perfusion rates, can change the amount of xenobiotic absorbed via inhalation and dermal absorption. As discussed previously, temperature has long been known to be an important influence on chemical reactions, including enzymatically controlled metabolic processes. The optimal temperature range for enzymatic reactions can, in fact, be quite narrow and thus be influenced by xenobiotic-induced thermoregulation. In addition, transport process, such as active transport in the gut can be temperature dependent. The net balance of these effects may be to change rates of detoxication or shift predominant metabolic pathways and products (for review, see Gordon, 2005).

To sum up, rodents and other small mammals are considered metabolic specialists, meaning the regulation of stable core temperature is intimately dependent on the maintenance of a high metabolic rate. In these species, a transient reduction in metabolic rate is quickly reflected in an abrupt decrease in core temperature. Large mammals rely more on vasomotor mechanisms to regulate heat loss. Regardless of body size, a state of homeothermy can only be achieved with a balance between heat production and heat loss (see Eq. (1)); however, larger species are less affected by a transient decrease in heat production as would be seen in a rodent. Because of their surface area:body mass ratio, rodents are better suited to elicit a regulated hypothermic response. This means that if a toxicant alters thermoeffector function to either generate hear or prevent heat loss, one should expect more rapid expression of hypothermia in smaller species. Moreover, the ability to elicit a regulated hypothermia will also be magnified as body mass is reduced. Hence, it should not be surprising that adult humans exhibit relatively small hypothermic responses

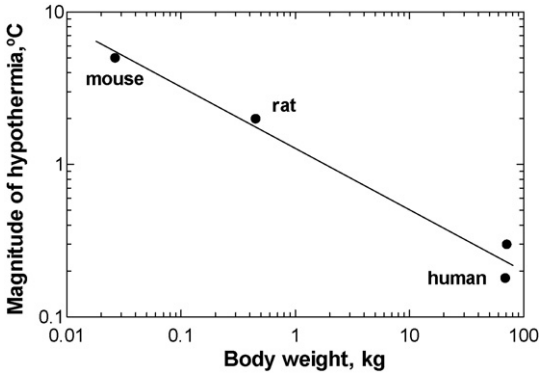

Fig. 6. Relationship between body size and hypothermic response to acute ethanol administration in mice, rats, and humans. Reprinted with permission from Gordon (2005).

when faced with a toxic insult. The data base is meager, much being collected from emergency room clinical reports (for review, see Gordon, 2005). There are a few clinical studies with controlled exposures to ethanol and other chemicals that shed additional light on the extrapolation of toxic-induced hypothermia from rodent to human (Fig. 6). The attenuation in hypothermic response to ethanol from mouse to human may exemplify the response to other toxicants that elicit hypothermia.

It is clear that a hypothermic response to a toxicant that has been well documented in mice, rats, and other small test species, is likely to be markedly attenuated or absent in adult humans. Large homeothermic species can be viewed as being essentially trapped in their *thermal milieu*, meaning that their thermal inertia is a major impediment to quickly lower core temperature in response to a toxic insult. Moreover, if large mammals do become hypothermic, the recovery to normothermia is slow and they would face an additional risk of remaining hypothermic for an extended period of time. It is postulated that the inability of relatively large species to lower core temperature must have been a driving force in the evolution of additional mechanisms to protect the animal from toxicants and other pathological insults when compared to smaller, thermally labile species (Gordon, 1996).

Why should toxicologists be so concerned about a hypothermic response in rodents that is rarely seen in adult humans? A better understanding of the thermoregulatory response in rodents and other species may improve the procedures for assessing the health and safety of toxicant exposure in humans. Assessing the risk of chemicals and drug safety relies on data collected from laboratory rodents. The exaggerated hypothermic response of rodents to a toxic insult may in fact lead the risk assessor to underestimate the toxicity of drugs and chemicals when extrapolating to humans (Gordon, 1991, 1996; Watkinson and Gordon, 1993). In other words, a moderate amount of hypothermia is protective to many types of toxic insults and the hypothermic response is attenuated with increasing body mass. Hence, a given dose of a toxicant that elicits a hypothermic response in a rodent would theoretically be more toxic in larger species that are unable to lower body temperature. This information could also be useful in the development of therapeutic treatments for poisoning. Finally, these studies may shed light on how birds, mammals and other species have evolved mechanisms to ameliorate the cellular damage from dietary and environmental toxicants as well as other types of insults.

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