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CHAPTER 5

Thermophysiological responses to hyperthermic drugs: extrapolating from rodent to human☆

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Abstract: This chapter focuses on the effects of hyperthermia on drug and chemical toxicity. In general, hyperthermia exacerbates the toxicity of many types of drugs and environmental toxicants. Using rodents to model the potential responses of humans to hyperthermic drugs is hampered by the unique differences in thermoregulatory ability and body mass. Because of their relatively large surface area:mass ratio, ambient temperature has a more profound influence on the potential hyperthermic effect of a drug in rodents. The relative increase in heat production (i.e., as a percentage of their basal metabolic rate) required to raise core temperature by 1°C will increase with a decrease in body mass. The thermoregulatory response to methylenedioxymethamphetamine (MDMA) is used to illustrate the differences in thermoregulatory responses of rats and humans to a hyperthermic drug. Overall, the interaction between ambient temperature and drug-induced changes in body temperature is critical in the evaluation of hyperthermic-induced toxicity in rodent models.

Keywords: temperature regulation; hyperthermia; extrapolation; MDMA

Introduction

Hyperthermia generally exacerbates the physiological and pathological responses of drugs, environmental contaminants, and other insults. Thermal kinetics provides a fundamental explanation of hyperthermia and drug efficacy. That is, the chemical reactions of all life processes depend on temperature, as expressed by the principles of the Arrhenius equation (Burton and Edholm, 1955). The Arrhenius equation is based on thermal kinetics, and states that the rate of chemical reactions increases exponentially with a rise in temperature (Fig. 1). Most molecular, cellular, and physiological processes have a positive temperature coefficient, meaning their activity increases in a manner similar to that predicted by the Arrhenius equation. Thermal biologists often use the Q_{10} to describe the effects of temperature. The Q_{10} of most biological processes ranges between 2 and 3, which equates to a doubling and tripling of the reaction rate with a 10°C increase in temperature.

Cellular and molecular mechanisms of toxicity generally have positive temperature coefficients (Gordon, 2005). Processes such as receptor binding, lipid peroxidation, metabolic deactivation of a toxicant, and oxidative phosphorylation generally

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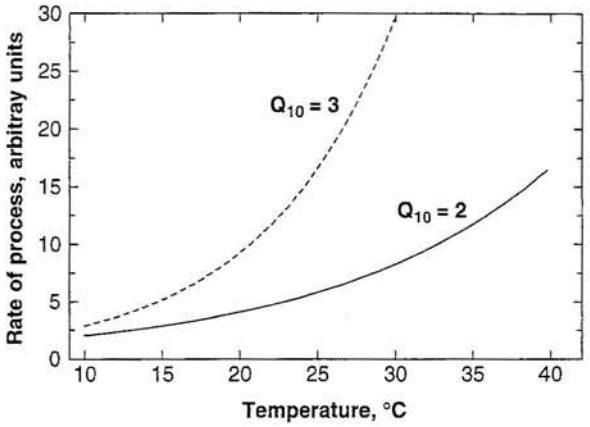

Fig. 1. A diagrammatic representation of the effect of temperature on the rate of a chemical reaction or physiological process. Theoretical functions show the effects of a doubling ($Q_{10} = 2$) or tripling ($Q_{10} = 3$) the rate of the process with a 10°C increase in temperature. Adapted from Schmidt-Nielsen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

increase with temperature but there are some notable exceptions. Insecticides such as DDT and pyrethroids that induce depolarization of nerve tissue and induction of some protective proteins, exhibit a negative temperature coefficient. In most cases, the toxic efficacy of a chemical is proportional to tissue temperature. Thus, in an environment where the thermoregulatory system is challenged such that a hyperthermic state exists, there will be increased susceptibility to a drug or toxic chemical.

This chapter has three main goals: review the literature on the role of hyperthermia on the toxic efficacy of drugs and xenobiotic agents, explore the mechanism of action of selected amphetamine-derivatives on the thermoregulatory system, and emphasize the importance of extrapolating thermoregulatory data from rodent to human.

Thermoregulatory profile and hyperthermic efficacy of drugs

Rodents have been and will continue to be a primary test species in the study of the toxicity of drugs and other chemical agents. Many investigators may not be fully aware of the interaction between ambient temperature, thermoregulatory requirements of rodents, and the hyperthermic efficacy of drugs and other agents. A thermoregulatory profile is essentially the interrelationship between ambient temperature, body temperature, skin temperature, and activity of thermoeffectors (Fig. 2). Measuring metabolic rate, evaporative water loss, and skin temperature (or skin blood flow) over a range of ambient temperature reveals a pattern of thermoeffector activity that is typical for rodents and most other mammals (Fig. 2). There is a range of ambient temperatures termed the *thermoneutral zone*, where metabolic rate is at or near basal levels. In this zone, temperature regulation is achieved by control of sensible heat loss, meaning without regulatory changes in metabolic rate or evaporative water loss. The control of body temperature in the thermoneutral zone is achieved with modulations in skin blood flow, which controls the rate of heat loss with no additional metabolic requirements. As ambient temperature decreases below the thermoneutral zone, the blood flow to the skin is minimal as a result of peripheral vasoconstriction. As ambient temperature decreases, metabolism must increase above basal levels by shivering and non-shivering thermogenesis in order for heat production to match heat loss to the environment. The ambient temperature where metabolic rate increases is termed the *lower critical temperature*. As ambient temperature decreases below the lower critical temperature, skin temperature falls passively but may increase with extreme cold exposure as a result of cold-induced vasodilation (CIVD) of the peripheral blood vessels. This is a protective response to keep

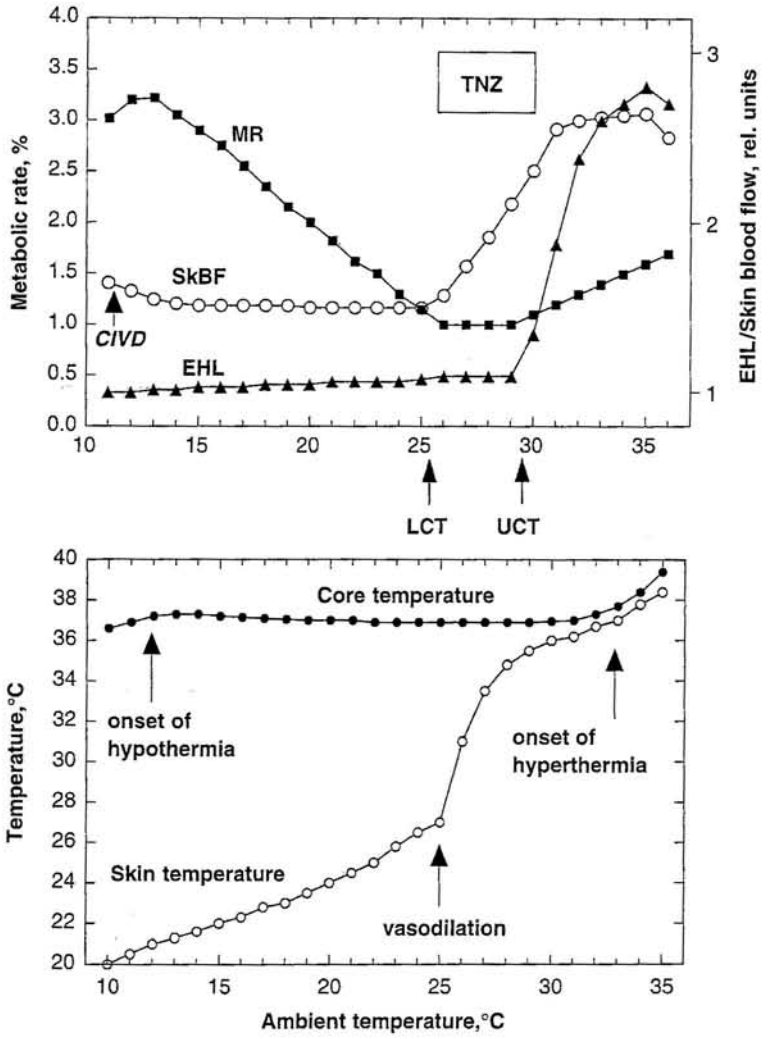

Fig. 2. General pattern of core and skin temperature and activity of autonomic thermoeffectors as a function of ambient temperature in a homeotherm. SkBF, skin blood flow; EHL, evaporative heat loss; MR, metabolic rate; LCT, lower critical temperature; UCT, upper critical temperature.

exposed tissues from freezing. Eventually, a temperature is reached where metabolic rate cannot maintain the pace of heat loss, and the animal becomes hypothermic.

As ambient temperature increases through the thermoneutral zone, skin blood flow increases and there is a disproportionate rise in skin temperature. However, if skin blood flow is maximal, skin temperature cannot increase above the internal core temperature. This means that as ambient temperature increases, the gradient between the skin and ambient temperature becomes smaller, thus limiting the rate of heat loss by convection, conduction, and radiation. Hence, at temperatures above the thermoneutral zone, evaporative heat loss mechanisms (i.e., panting, sweating, saliva grooming) must be activated to maintain thermal balance. This ambient temperature is termed the *upper critical temperature.* It is also identified with the point where core temperature and metabolism begin to rise (see IUPS Thermal Commission, 2001). At around the point of the upper critical

temperature, skin temperature has increased to a level that is just below core temperature, reflecting maximal redistribution of warm blood from core to the periphery. With further increase in ambient temperature, skin and core temperature parallel each other until the point of thermoregulatory failure. At this point, evaporation is ineffective to maintain sufficient heat loss and core temperature spirals upward leading to hyperthermic death.

It is obvious that the hyperthermic efficacy of a drug will be exacerbated when rodents are housed at a warm temperature that exceeds thermoneutrality. But why should researchers studying the efficacy and responses of hyperthermic drugs be concerned with the characteristics of the thermoregulatory system below thermoneutrality? Due to their small size, rodents have a relatively large surface area:mass ratio, meaning that they lose body heat faster and must rely more on a high metabolic rate rather than adjustments in peripheral vasomotor tone to thermoregulate below the lower critical temperature. Mice and rats have a lower critical temperature of 28° to 31°C, which is notably much warmer than the standard temperature for housing in most laboratory settings. In other words, under standard conditions they are essentially cold stressed and thermoregulate by maintaining a metabolic rate above basal levels. Hence, in studies where the efficacy of hyperthermic drugs is studied at standard room temperature, the mouse and rat are in an environment that is relatively cool and this will impact on the thermoregulatory response to a drug. Most importantly, it will affect the manner in which one wants to extrapolate the effects of the drug to that of a human (see below).

One can argue that rodents maintained under standard housing conditions of 22°C are able to acclimate to the cool conditions and therefore are not "stressed". However, when one puts rats, mice, and other rodents in a temperature gradient, they select temperatures associated with metabolic thermoneutrality (approximately 28° to 30°C), a temperature that is warmer than that of their housing conditions (Gordon, 1993). Hence, one can infer from this behavior that rodents are often housed under conditions that are likely to be a mild cold stress. Of course, the housing conditions, including number of animals per cage, bedding type, air flow, and other factors will affect the rodent's thermoregulatory response under so-called standard conditions. For example, wood shaving bedding that allows for burrowing will minimize the effects of ambient temperature on heat loss while a wire-screen floor accelerates heat loss. Groups of mice housed in cages with wood shavings maintain a significantly warmer core temperature during the day than that of mice housed on wood chip bedding which does not allow for burrowing (Gordon, 2004).

Ambient temperature and potential hyperthermic response

Whether a toxicant will cause an increase, decrease, or have no effect on body temperature will depend largely on ambient temperature and the thermoeffector systems affected (Table 1). The potential changes in temperature, indicated by the number of arrows in each block of the table, is dependent on whether the animal is housed in a cool, warm, or thermoneutral environment. For example, a drug that blocks metabolic thermogenesis will manifest the most effective change in body temperature when exposure occurs at a relatively cool ambient temperature. A toxicant that induces peripheral vasoconstriction, thus restricting blood flow and heat loss from the skin will have relatively minor effects in a cold environment because the animal is in a state of peripheral vasoconstriction but would lead to hyperthermia in a thermoneutral and warm environment. On the other hand, an agent that causes peripheral vasodilation would be mostly ineffective in a warm environment because skin blood flow is already elevated and an additional vasodilatory action should have little effect on total heat loss. Blocking salivation in rodents or sweating in humans would have little effect in the cold but would lead to dramatic hyperthermia if the blocking agents are administered in a warm environment.

If the toxicant impairs one thermoeffector without affecting CNS thermoregulatory control, then one would expect the animal to utilize other thermoeffectors to maintain thermal homeostasis. For example, if skin blood flow was stimulated in a

Table 1. Relative hyperthermic (↑) and hypothermic (↓) effects of a toxicant or drug that stimulates or blocks thermoeffectors at cool, thermoneutral, and warm ambient temperature

	Thermogenesis increased	Skin blood flow increased	Evaporation increased	Thermogenesis blocked	Skin blood flow blocked	Evaporation blocked
Cool temperature	↑	↓	↓	↓		
Thermoneutral temperature	↑	↓		↓	↑	↑
Warm temperature	↑	↓		↓	↑	↑

Size of arrow indicates relative magnitude of change in core temperature. Adapted from Gordon (2005).

cold environment, then metabolic thermogenesis could increase to counter for the increased heat loss. Stimulation of metabolism in a warm environment, such as, occurs by exposure to chemical agents that uncouple oxidative phosphorylation, is accompanied by a marked increase in evaporation (Wood et al., 1983). Overall, these are idealized situations and drugs and toxicants generally affect the function of more than one thermoeffector system.

Patterns of drug toxicity as function of temperature

Fuhrman and Fuhrman (1961) first developed a general scheme to describe the toxicity of drugs as a function of ambient temperature (Fig. 3). The term "toxic potency" in the ordinate can describe both lethal and non-lethal endpoints. In general, there are three basic functions that describe the toxicity of drugs in homeotherms exposed over a wide range of ambient temperatures: (A) a V- or U-shaped function with minimum toxicity (i.e., high LD_{50}) at a narrow range of temperatures usually below the metabolic thermoneutral zone and increased toxicity as temperature increases or decreases from the nadir of the curve; (B) a linear function with toxicity increasing with temperature; and (C) a function with a constant toxicity over a wide range of cool ambient temperatures and then sudden increase in toxicity at a threshold temperature around or above the upper portion of the thermoneutral zone. The Type C response is questionable in the sense that if temperature is reduced to sufficient levels, a toxicant would eventually respond in a Type A response. Like the efficacy of many drugs, the toxicity of xenobiotics can follow one of the three responses depicted in Fig. 3. In general, the *in vivo* toxicity of mice and rats as a function of temperature exhibits the Type A response (V-shaped). Examples of a Type A response include chlorpromazine and toluene. Toxicants such as ethanol and dinitrophenol display a Type B response, while DDT shows a Type C response (Fig. 3). Whether a toxicant fits the Type A, B, or C function is dependent in part on the mechanism of action (Weihe, 1973). For example, agents that are sympathomimetics and stimulate metabolic thermogenesis would be expected to take on a Type B response. That is, the effectiveness of a compound to cause a dangerous elevation in core temperature, such as oxidative phosphorylation uncouplers, would increase with ambient temperature.

The Type A response is likely the most relevant function for most drugs and toxicants. The V- or U-shaped function is thought to be a result of agents that interfere with the CNS control of body temperature. Although Weihe (1973) suggested that Type A drugs would not be involved with a re-setting of the thermoregulatory set-point, the evidence with many toxicants would suggest otherwise. This function is a result of the combined

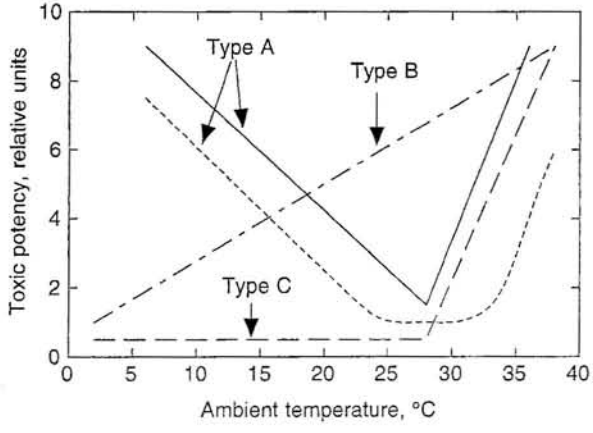

Fig. 3. General pattern of drug and chemical toxicity as a function of ambient temperature. Adapted from Fuhrman and Fuhrman (1961).

effects of the toxicant and ambient temperature on thermoregulatory control. That is, many toxic chemicals induce a regulated hypothermic response in rats and mice. There is an ideal range of ambient temperatures where the treated rodent can maintain a moderate hypothermic core temperature (Gordon, 2005). If ambient temperature changes above or below optimal levels, the thermoregulatory effectors are overwhelmed and are unable to maintain core temperature, possibly resulting in death from thermoregulatory failure.

Core temperature: its effect on magnitude versus duration of toxicity

The response or sensitivity to a toxicant is characterized by both the magnitude and length of time that the toxicant persists in the body (Doull, 1972). In most cases, hyperthermia is going to reduce the duration but increase the magnitude of toxicity. That is, when body temperature is raised, the mechanism(s) responsible for metabolizing and excreting the toxicants are going to be accelerated because the temperature coefficients for these processes are positively affected by temperature. Doull (1972) developed a general prediction of the response of biological systems to toxic levels of chemicals that can be applied to most drugs and toxic chemicals. "Temperature is directly correlated with the magnitude and inversely correlated with duration of drug response in biological systems". In other words, while the concentration of a toxicant will persist longer during hypothermia, the toxicity of the agent is reduced. This is a favorable situation for the efficacy of chemotherapeutic agents and the treatment of cancer because it allows for one to use lower doses of chemotherapeutics by raising the local temperature of the tissue to be treated (Falk and Issels, 2001).

The tenet of Doull's was stated over 30 years ago and remains a critical issue in toxicology and pharmacology. The magnitude and duration of a toxicity has a bearing on experimental and clinical toxicological studies. First, rodents are the primary species for toxicological studies and their thermoregulatory system is extremely sensitive to toxic insults. Their integrated thermoregulatory responses will have a marked impact on the pharmacokinetics and overall toxicity of the chemical or drug. Second, if hyperthermia exacerbates toxicity and hypothermia affords protection to toxicants, then this should have important implications in the way in which humans and domestic species are treated following episodes of poisoning (Gordon, 2005).

Extrapolating from rodent to human

An assumption of some degree of similarity between rodents and humans is the mainstay of biomedical research. Drug development and safety, toxicology, and other responses are studied in rodents with the notion that the effects can be extrapolated to that of humans. There can be tremendous uncertainty in the extrapolation process. Thermoregulatory sensitivity is a critical factor in the extrapolation from rodent to human that is often not considered. It is extremely important in the understanding of the hyperthermic drugs.

In addition to ambient temperature (e.g., Table 1), it is also important to consider the species' body mass when attempting to predict how a change in thermoeffector function will affect the control of body temperature. For example, species such as rodents with a relatively small body mass and large surface area:body mass ratio rely mostly on metabolic thermogenesis to thermoregulate, whereas peripheral vasomotor tone becomes more critical in species with large body mass. This also means that the amount of heat production needed for a given elevation in core temperature is going to increase with a reduction in body mass. This can be best illustrated by a plot of species body mass versus the amount of heat from microwave radiation needed to raise core temperature by 1°C (Fig. 4). In these studies, mice, rats, golden hamsters, and rabbits were exposed to RF radiation for 90 min while maintained at ambient temperatures of 20 and 30°C. An ambient temperature of 20°C is relatively cool for the mouse, hamster, and rat but near thermoneutral for the rabbit. A temperature of 30°C is near thermoneutrality for the rodents but a mild to moderate heat stress for the rabbit.

At both ambient temperatures there was clear inverse relationship between body mass and the

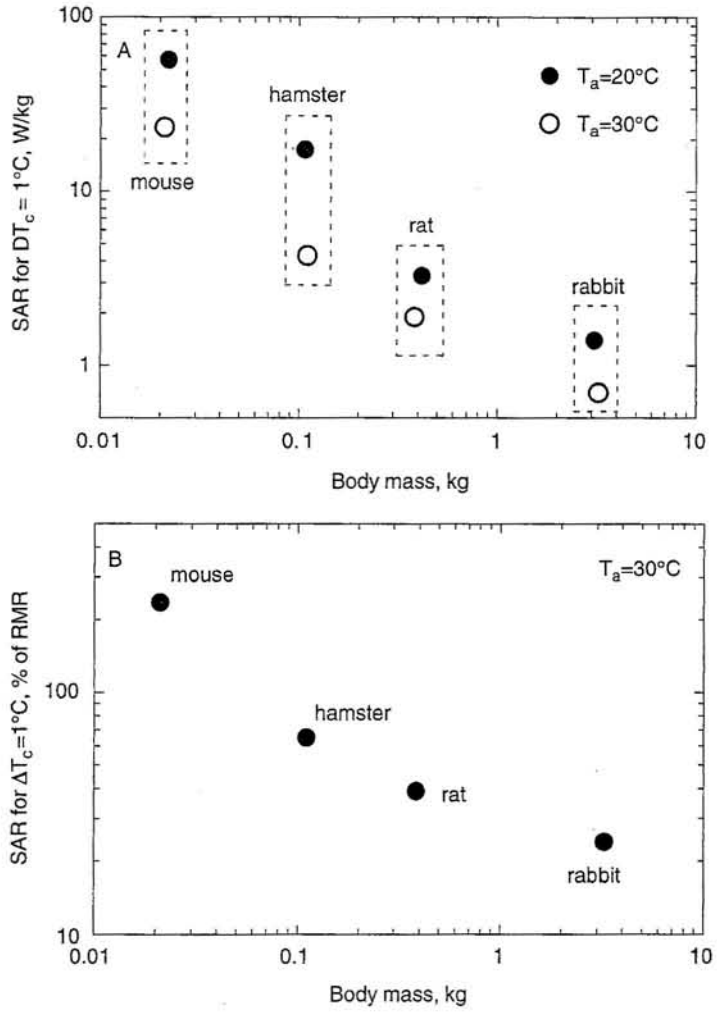

Fig. 4. (A) Relationship between body mass and the specific absorption rate (SAR) required to raise core temperature by 1°C in various species exposed to radiofrequency radiation for 90 min at ambient temperatures of 20 and 30°C. (B) Calculation of SAR as percentage of resting metabolic rate (RMR) required to raise core temperature by 1°C at an ambient temperature of 30°C. Data adapted from Gordon et al. (1986a, b).

amount of RF energy needed to raise core temperature by a given amount. It is important to note that the dosimetry units for exposure to RF radiation are the same as for metabolic rate normalized to body weight (i.e., W/kg). This provides an ideal means of comparing thermoregulatory responses across species. By calculating the SAR as a percentage of the species' metabolic rate at an ambient temperature of 30°C, one sees a marked decrease in the relative heat production that would be needed to raise body temperature with increase in body mass (Fig. 4).

Body mass also affects the manner in which heat is dissipated by physiological mechanisms. The contribution of peripheral vasomotor tone for heat dissipation is also linked to body mass. Assessing if the drug or toxicant causes vasodilation or vasoconstriction is a common approach in rodent studies with the intent that the vasomotor response can be extrapolated to that of a human response.

To make comparisons in the vasomotor responses of rodents and humans, one must also consider the impact of body size. Phillips and Heath (1995) utilized infrared thermography in a quantitative study of the impact of body mass on the role of peripheral vasomotor tone in thermoregulation. They noted that a small mammal such as a mouse or rat, by having a relatively large surface area:volume ratio, are "metabolic specialists". That is, homeotherms of small size rely on changes in their metabolic rate as a primary means to regulate body temperature. Large animals do not change metabolic rate as much with changes in ambient temperature and thermoregulate by controlling their surface temperature. The vasomotor index, a value reflecting the ability of an animal to regulate heat exchange through control of its skin temperature, was shown to be directly proportional to body mass. For example, the vasomotor index of a 80 kg human was predicted to be more than 6-fold greater than that of a 300 g rat. Could this mean that a vasomotor effect of a drug in a small mammal is magnified in a larger species that relies more on peripheral vasomotor tone to thermoregulate? Further work in comparative thermoregulatory responses to drugs and toxicants will help answer this question.

One should not infer from this discussion that rodents are better adapted to resist heat stress. In fact, the opposite is true when viewing heat resistance in terms of exposure to high ambient temperatures and/or radiant heat loads (Adolph, 1947). That is, due to their large surface area:mass ratio, mice and rats heat up quickly when exposed to high ambient temperatures relative to that of large mammals. With a smaller size, rodents must quickly dissipate heat by evaporation to prevent an explosive rise in core temperature. Rodents are capable of dissipating heat by evaporation through grooming of saliva and urine on their fur but they cannot maintain this response for long periods of time.

Recovery from acute elevations in core temperature is another important facet of extrapolation that is relevant to the study of hyperthermic drugs and toxic agents. The extrapolation of studies pertaining to lethal body temperature in mammals should be less problematic because the denaturation of proteins and cellular breakdown is universal, regardless of species. Overall, lethal body temperature is nearly equal among eutherian mammals although there are some peculiar species differences (Adolph, 1947). On the other hand, if one is interested in exploring the effects of transient exposure to high body temperatures, then it is important to understand that rodents will recover much faster than humans and other large species when exposed to near lethal heat stress. In the study of the thermoregulatory effects of exercise, it is recognized that small mammals (i.e., <1 kg) are capable of dissipating most of the heat burden by non-evaporative mechanisms because of their large surface area:mass relationship (Taylor, 1977). In large mammals (>10 kg), non-evaporative mechanisms are insufficient and greater reliance is placed on sweating to dissipate the heat load from exercise. This characteristic of body mass is important to consider in the study of hyperthermic drugs and the extrapolation of their effects from rodents to humans.

To summarize, in the extrapolation from rodent to human, one would expect the required increase in heat production to raise core temperature as a percentage of the basal metabolic rate would decrease. Small rodents are as not in much danger of overheating from a drug when exposed at a standard room temperature because they can easily dump heat by passive mechanisms due to their large surface area:volume. Humans are well adapted to dissipate heat by evaporation and vasomotor mechanisms but can overheat quickly if a drug or toxicant impairs heat loss mechanisms. These relationships are simplified and could certainly be affected by a variety of biological and environmental factors. One must be cautious in extrapolating a potential thermoregulatory effect from rodent-to-human or human-to-rodent.

Methylenedioxymethamphetamine (MDMA)

Rodent response

MDMA (street name: Ecstasy) is an ideal drug for discussion in this chapter for several reasons: (i) there is a substantial database on its thermoregulatory effects in both experimental animals and humans; (ii) it is a heavily used drug of abuse with

thorough studies on its use and abuse; (iii) it is also considered a potential candidate for therapeutic treatment of psychoses; and (iv) overdose exposures to MDMA and resultant mortality has been attributed to its disabling of thermoregulatory mechanisms and death by hyperthermia.

MDMA stimulates the release of serotonin in the CNS resulting in a euphoric-like state. Serotonergic pathways in the CNS are also critical in the regulation of body temperature. It was recognized years ago that the abuse of MDMA in humans while engaged in dancing in clubs with poor ventilation and warm temperatures led in some cases to severe cases of hyperthermia and occasional deaths (Henry et al., 1992). The thermoregulatory effects of this drug in rats and mice have since been extensively studied.

Our laboratory was one of the first to study the thermoeffector responses of rats when dosed with MDMA at different ambient temperatures (Gordon et al., 1991). We found that the hyperthermic effects of MDMA were markedly dependent on ambient temperature (Fig. 5A). Rats and mice become hypothermic with MDMA given at relatively cool ambient temperatures, whereas the same dose give at warm temperatures results in hyperthermia. For example, a dose of 30 mg/kg MDMA in the rat led to hypothermia at 10°C, no effect at 20°C, and near lethal hyperthermia at 30°C (Fig. 5A). It is important to note that these same ambient temperatures have little effect on baseline core temperature of the rat. The hypothermic and hyperthermic effects are attributed to the combined effects of MDMA on heat production and heat loss. MDMA led to significant elevation in metabolic rate at ambient temperature of 20 and 30°C and evaporative water loss at ambient temperatures of 10, 20, and 30°C when housed in a metal chamber. MDMA also caused vasoconstriction regardless of ambient temperature (see below). MDMA stimulates motor activity due to its effect on serotonin release but its hyperthermic effects were only manifested at warmer temperatures. Vasoconstriction of skin blood flow in the cold will have little impact on a potential hyperthermic effect of a drug but the increase in evaporation was more likely a key factor explaining the hypothermia in the cold. At 20°C the increase in metabolic rate was essentially balanced by the increase in evaporative water loss resulting in no change in core temperature. At 30°C, the increase in metabolic rate combined with tail vasoconstriction was more than enough to overwhelm any heat loss from evaporation; the net result being near lethal hyperthermia.

The floor of the cage was critical in the thermoregulatory outcome of MDMA. When kept at room temperature in a cage with a metal floor that easily conducts heat away from the rat, the hyperthermic effects were less apparent. On a floor of low thermal conduction such as plastic or wood shavings, the hypothermic effects were most profound (Gordon and Fogelson, 1994). One of the most amazing effects on thermoregulation in the rat was the inability of the rat to vasodilate its tail in spite of encountering lethal hyperthermia (Fig. 5B). For example, 3 h after dosing with 20 mg/kg MDMA at ambient temperature of 22°C, the core temperature is at near lethal level of 41.5°C while tail skin temperature was 2.2°C below that of animals dosed with saline. By comparison, rats exposed to a hot ambient temperature of 37°C for 1 h had a core temperature of 40.5°C and a tail skin temperature of 31.7°C, reflecting a marked rise in skin blood flow. Note that an ambient temperature of 26° to 28°C is considered the threshold ambient temperature for tail vasodilation in the rat (Gordon, 1993). In rabbits, MDMA elicits profound vasoconstriction of blood flow to the ears, resulting in hyperthermia (Pedersen and Blessing, 2001). On the other hand, there was no indication of vasoconstriction of skin blood flow in humans administered MDMA (see below).

Jaehne et al. (2005) have recently measured behavioral thermoregulatory responses of rats in a temperature gradient when dosed with MDMA. They found that behavioral thermoregulatory mechanism to regulate body temperature were apparently unaffected by MDMA administration. For example, rats given MDMA and maintained in a warm environment quickly selected cool temperatures and brought their core temperature back to normothermic levels. Rats made hypothermic by administering MDMA and exposed in a cold environment quickly selected warmer temperatures and brought core temperature back to

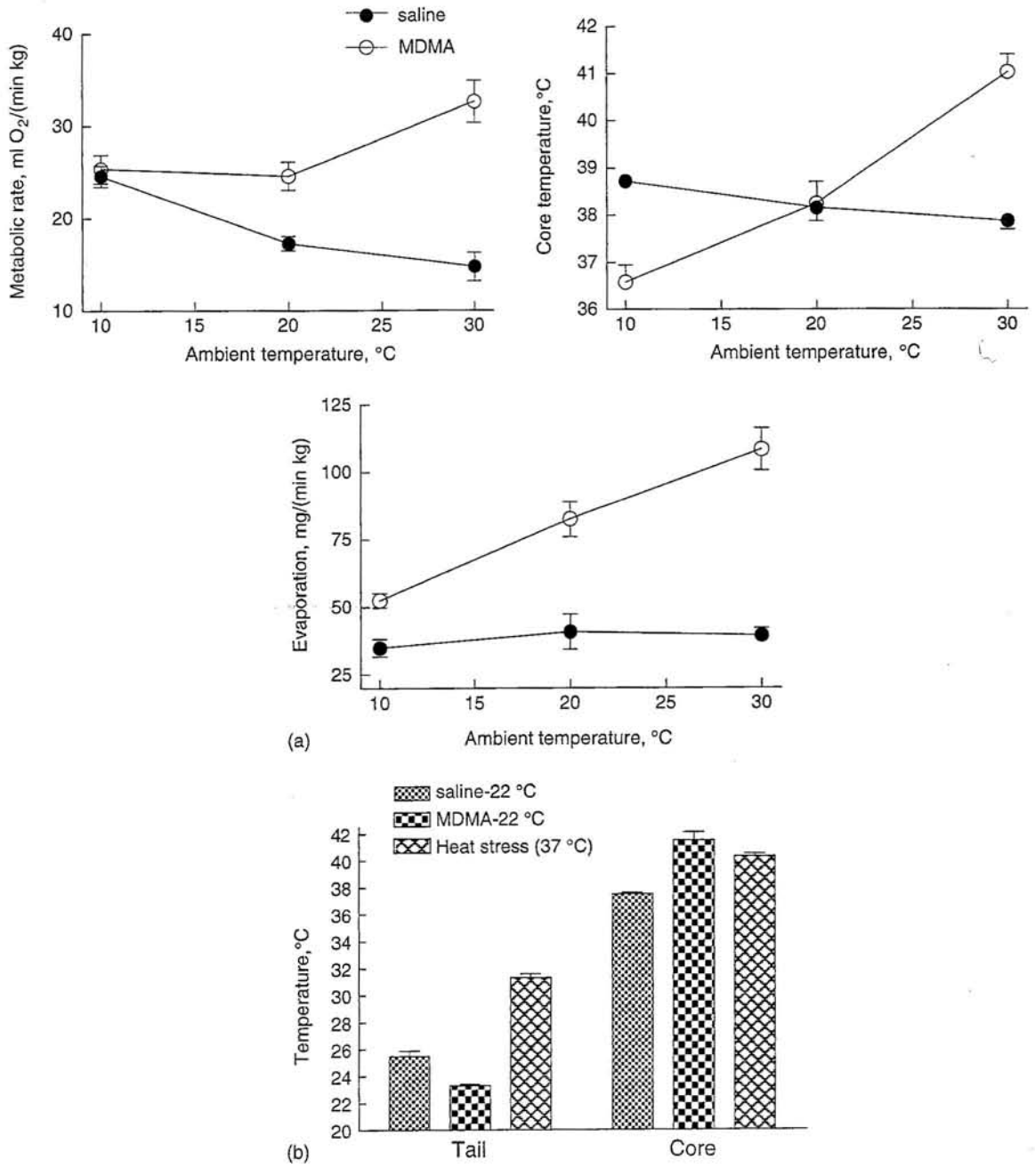

Fig. 5. (a) Effect of ambient temperature on the metabolic rate, evaporative water loss, and body temperature of rats dosed intraperitoneally with 30 mg/kg MDMA. Relationship between core and tail skin temperature in the rat dosed with 20 mg/kg MDMA when housed at an ambient temperature of 22°C. Also shown is the effects of heat stress (1 h at 37°C ambient temperature) on tail and core temperature. (b) Note relatively cool tail skin temperature in spite of hyperthermic body temperature in MDMA-treated rats as compared to warm tail (i.e., vasodilation) in heat-stressed rats. Data adapted from Gordon et al. (1991).

normal. Hence, autonomic thermoeffectors are clearly dysfunctional in MDMA-treated rats whereas behavioral thermoregulation appears to be unaffected. The response of the rat in thermal gradient provides an interesting contrast to the perceived thermal environment of humans given MDMA (see below).

Human response

In its use as a recreational drug MDMA is often ingested in crowded dance clubs ("raves") that can be quite warm. The primary toxic effect of MDMA in humans is hyperthermia with core temperatures as high as 43°C reported (Henry et al., 1992). It is clear that the thermoregulatory effects of MDMA combined with the impact of heat production from dancing in a warm environment will interact to compound the hyperthermic effects of MDMA. Until recently, there was little known on the thermoregulatory effects of MDMA in humans. Freedman et al. (2005) reported on the effects of MDMA ingestion in human volunteers (previous MDMA users) maintained at a relatively cold (18°C) and warm (30°C) ambient temperature. Core temperature measured by a radiotelemetric pill, metabolic rate, sweating, skin temperature, and subjective feelings of warm and cold were assessed. Three hours after ingesting 2 mg/kg MDMA, core temperature began to rise above control levels, increasing by 0.6 and 0.3°C in subjected exposed to warm and cold ambient temperatures, respectively (Fig. 6). The rise in temperature was associated with a mild rise in metabolic rate in the warm temperature and a sharper rise in metabolism in the cold. Interestingly, there was no indication of vasoconstriction as measured by skin temperature. While exposure to the warm temperature elicited a sweating response, it is interesting to note that sweating in the MDMA-exposed group was delayed by approximately 30 min in subjects given MDMA in the warm temperature. Subjects in the heat given MDMA had a greater perception of warmth compared to controls but their autonomic effectors (sweating, vasodilation) were apparently not activated to lower core temperature.

The response of humans and rodents to MDMA is similar in that autonomic effectors are activated to raise core temperature and there appears to be a normal behavioral sensation of warm temperatures. On the other hand, there are distinct differences between species. The effects of ambient temperature on the thermoregulatory response to MDMA were not as profound in humans. Rodents become hypothermic in the cold while the human subjects exposed to a cold environment that was sufficient to increase metabolic rate became hyperthermic with MDMA ingestion. Moreover, peripheral vasoconstricition is a key thermoeffector in the rat exposed to MDMA whereas this response seemed to be lacking in the human subjects. These comparisons have to be viewed with caution in view of the differences in doses and route of administration and the previous exposure to MDMA in the human subjects.

Interaction between hyperthermia and drug toxicity

Toxic mechanisms attenuated by hypothermia

Hypothermia affords protection to a variety of drugs and toxicants while hyperthermia augments toxicity. Systemic toxicological studies have shown that mild hypothermia improves the recovery of organ function and chances of survival following exposure to a variety of toxicants but there is relatively little known on the mechanisms of action (Gordon, 2005). Hyperthermia augments toxicity of most chemicals and drugs. Based on the Arrhenius relationship, it is reasonable to assume that hypothermia is most likely protecting tissues by slowing the rate at which the toxicant exerts its damaging effects at the cellular and intracellular level. That is, the kinetics of cell death as a function of temperature and toxicant exposure is often studied using an Arrhenius plot. When the relationship between temperature and cell killing is linear then it could be assumed that the toxicant follows a Arrhenius type of temperature dependence (see Gordon, 2005). On the other hand, a sudden departure from linearity would indicate a non-additive cytotoxic of the chemical with temperature. For example, Arrhenius plots for *in vitro* cell inactivation by the genotoxicants bleomycin and paraquat show a distinct, non-linear break while other anti-cancer agents show a linear

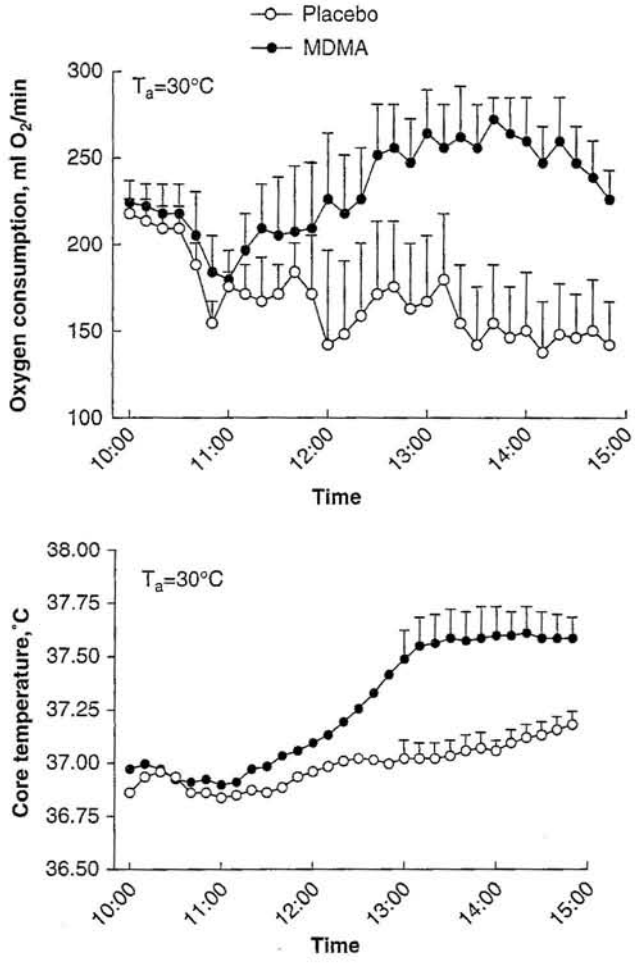

Fig. 6. Time-course of core temperature and metabolic rate in human volunteers administered MDMA. Data modified from Freedman et al. (2005).

temperature-dependent cytotoxicity (Takayoshi et al., 1987). A non-linear Arrhenius plot could result from simultaneous activation of the toxicant of two or more processes that have different activation energies.

There are other mechanisms to consider that go beyond Arrhenius type responses to explain the beneficial effects of mild hypothermia and damaging effects of hyperthermia. Recently, there have been numerous studies designed to understand the mechanisms of hypothermic protection to the CNS of rodents treated with substituted amphetamines such as MDMA (O'Callaghan and Miller, 2002). As discussed earlier, methamphetamines are drugs of abuse and are also well-known neurotoxicants that cause depletions in striatal levels of dopamine and serotonin. The database for the methamphetamines is considerable and a review of the mechanism of action of the amphetamines and their interaction with body temperature is relevant to understand the mechanisms of hypothermic protection exhibited by many of the other drugs and xenobiotics.

Blocking the hyperthermic effects or allowing the rodents to become hypothermic imparts remarkable protection on the neurotransmitter-depleting actions of the amphetamines. These observations have spurred numerous investigations on the mechanisms

Table 2. A summary of studies with plausible mechanisms to explain how hypothermia protects tissues in the CNS from pathological insults

Insult	Mechanism	Reference
Amphetamine toxicity	Attenuated 5-HT and DA release from striatum	Bowyer et al. (1992), Malberg et al. (1996)
Amphetamine[a] toxicity	Attenuated DA release and GFAP accumulation in striatum	Miller and O'Callaghan (1994)
Amphetamine toxicity	Prevented Ca^{+2} accumulation in nigrostriatum	Corbett et al. (1990)[a]
CNS ischemia	Reduced free radical formation	Globus et al. (1995)
CNS ischemia	Blocked translocation of PKC in striatum	Cardell et al. (1991)
CNS ischemia	Reduced ATP depletion in hippocampus	Zeevalk and Nicklas (1993)
CNS ischemia	Reduced glutamate and glycine release in hippocampus	Illievich et al. (1994)
Trimethyl tin	Reduced accumulation of GFAP in hippocampus	Gordon and O'Callaghan (1995)
CNS ischemia	Reduce oxidative stress metabolite (MDA) production	Katz et al. (2004)

Key: 5-HT, serotonin; DA, dopamine; PKC, phosphocreatine kinase C; GFAP, glial fibrillary acid protein; MDA, malondialdehyde.
[a]Various substituted amphetamines studied.

of amphetamine-induced striatal dopamine depletion and the protection afforded by hypothermia (e.g., Malberg and Seiden, 1998). For example, after treating rats with MDMA there is a marked depletion of serotonin in the striatum, frontal cortex, and other CNS sites when measured 14 days after treatment (Malberg et al., 1996). Preinjection with ketanserin (KET) or α-methyl-*p*-tyrosine (AMPT) with MDMA causes a profound hypothermia and no depletion in serotonin levels. Blocking the hypothermia in MDMA-treated animals given KET or AMPT led to marked serotonin depletion suggesting that the reduction in core temperature afforded neuroprotection by preventing loss of neurotransmitters. On the other hand, fluoxetine given with MDMA also blocks serotonin depletion but this combination of drugs has no effect on body temperature. Overall, these experiments serve as just one example to illustrate the difficulty in separating the protective actions of a drug or toxicant that is directly dependent on a thermoregulatory response or completely independent of body temperature. Researchers studying the methamphetamines have been frustrated by the difficulty to separate a true neuroprotective mechanism of a chemical treatment from the general protective effects of hypothermia. This frustration was best summed up in a review on the mechanisms of amphetamine neurotoxicity by O'Callaghan and Miller (2002) "Although painful to consider, what these data suggest is that the results of all 'mechanistic' studies of amphetamine neuropharmacology or neurotoxicity are compromised unless temperature can be ruled out as a contributing factor".

There has also been a resurgence to understand how hypothermia affords protection to the CNS following stroke as well as other types of traumatic brain injuries (Dietrich, 1992; Ginsberg and Busto, 1998). Like the amphetamine studies, there has been tremendous success in connecting a specific cellular or molecular endpoint that is manifested with hypothermia but it has been difficult to identify whether the mechanism is a direct result of hypothermia (Table 2). The potential impact of hyperthermia on the recovery from CNS ischemia is extremely relevant because fever is commonly observed in stroke patients and has been shown to be a key factor that impedes recovery (Ginsberg and Busto, 1998; Hanchaiphiboolkul, 2005). Many of the protective effects of hypothermia can likely be applied to toxicological studies because many toxicants exert the same sequelae as that seen from ischemia, including lipid peroxidation, free radical formation, Ca^{+2} imbalance, and many others (Stohs and Bagchi, 1995; Gultekin et al., 2000). The stroke and ischemia literature may well be helpful to the endeavors of toxicologists and pharmacologists to understand the protective role of hypothermia.

Reactive oxygen species (ROS)

Formation of ROS and the protective role of hypothermia has emerged as a key mechanism to understanding the mechanisms of toxicant-induced

degeneration of the CNS and other tissues and organs (Halliwell, 1992). ROS cause damage to proteins, lipids, and nucleic acids leading to cell damage and death. Slikker et al. (2001) found that the percentage of surviving Chinese hamster ovary cells subjected to oxidative stress by exposure to hydrogen peroxide was inversely related to the temperature of incubation. This is not surprising in view of the studies that have shown how hyperthermia accentuates lipid peroxidation and free radical formation (Table 3). Furthermore, it was shown that reducing the incubation temperature led to greater expression of *bcl-2*, an anti-apoptotic protein that affords marked protection against oxidative stress. This experiment shows how hypothermia protects cells by more than just a simple Arrhenius effect and hyperthermia would most likely suppress the protective effects (Fig. 7). That is, lower temperatures induce and higher temperatures suppress the expression of a protein that is critical for cell survival. In this scheme, hyperthermia accentuates the activity of damaging processes that have a positive temperature coefficient (e.g., lipid peroxidation, ROS formation). Hypothermia activates expression of *bcl-2* that further protects cells from oxidative stress.

Table 3. A summary of studies with plausible mechanisms of how hyperthermia exacerbates the toxicity of drugs and other agents

Insult	Mechanism	Reference
MDMA	Accentuate production of lipid peroxidation products in liver	Carvalho et al. (2002)
Carbon monoxide	Accentuate production of lipid peroxidation products in brain	Kudo et al. (2001)
Turpentine fever	Accentuated lipid peroxidation (MDA) in kidney and liver	Brzezinska-Slebodzinska (2001)
Aging	Accentuates liver peroxidation and reduce catalase activity	Ando et al. (1997)
L-ephedrine	CNS neurodegeneration	Bowyer et al. (2001)

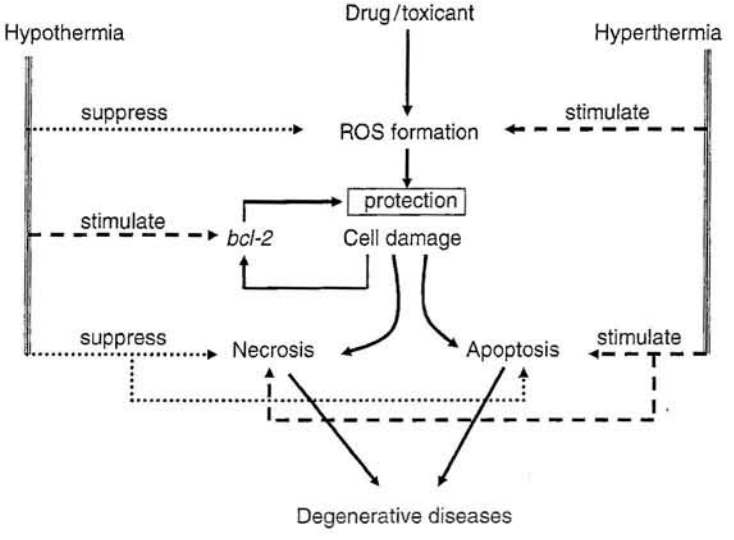

Fig. 7. A general mechanism of how hypothermia and hyperthermia can modulate the toxic-induced cell damage and the manifestation of neurodegenerative disease. Damaging processes with a positive temperature coefficient or $Q_{10}1.0$, including formation of reactive oxygen species (ROS), are exacerbated with an increase in temperature. Expression of proteins that protect cells from ROS formation such as *bcl-2* is a process that has a negative temperature coefficient and is activated with hypothermia. Hypothermia suppresses cell necrosis and apoptosis. Adapted from Gordon (2005) also see Slikker et al. (2001).

Conclusions

Hyperthermia is going to exacerbate the toxicity of many drugs and other chemicals. Ambient temperature and species mass are critical issues in the study of the toxicity of drugs and other chemicals in animals that are hyperthermic. The efficacy of a drug to raise body temperature is obviously dependent on ambient temperature. But the specie's body mass will also impart a significant impact on the effect of the drug on body temperature. One must be aware that, while rodents are excellent thermoregulators, they are more thermally labile when their thermoregulatory system is faced with a toxicant or other insult. Hyperthermic responses to a drug could be more transient and difficult to see in rodents compared to larger species. Radiotelemetry is thus the ideal method for monitoring the thermoregulatory responses to hyperthermic drugs in small rodents. Moreover, using laboratory rodents to model the responses of hyperthermic drugs in humans requires a firm understanding of comparative physiology and principles of scaling.

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