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BRIEF COMMUNICATION

Thermoregulatory Responses in Mice Following Acute Administration of Principal Nitrogenous Excretory Substances[1]

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GORDON, C. J. *Thermoregulatory responses in mice following acute administration of principal nitrogenous excretory substances.* PHARMACOL BIOCHEM BEHAV 31(3) 699–703, 1988.—This study was designed to assess the effects of some key excretory nitrogenous substances on body temperature and selected ambient temperature (T_a) in the mouse. In the first experiment, a dosage-response curve was developed to assess the effects of urea, creatinine, and ammonium chloride on colonic temperature at a T_a of 20°C. All three substances elicited a drop in body temperature at a critical dosage. The threshold dosages were 3280 mg/kg for urea, 1279 mg/kg for creatinine, and 365 mg/kg for ammonium chloride. In a second experiment the selected T_a was monitored using a temperature gradient system. Mice were injected with dosages of the nitrogenous substances that had previously been shown to cause hypothermia at a T_a of 20°C. Urea and ammonium chloride had no significant effect on the selected T_a nor on the colonic temperature after 90 min in the temperature gradient. Creatinine elicited a slight lowering of the selected T_a but had no effect on colonic temperature. The thermoregulatory responses to extremely toxic dosages of the nitrogenous substances appear to be quite dissimilar to that when animals are treated with xenobiotic compounds.

Colonic temperature Behavioral thermoregulation Azotemia Urea Creatinine Ammonium chloride

DURING renal failure there is a rapid increase of many nitrogenous substances in the blood including urea, creatinine, uric acid, guanine, and several other compounds (12). The build-up of these compounds during renal failure is termed uremia and is associated with a variety of pathophysiological conditions including dysfunction of the neural, cardiovascular, and locomotor systems (10,12). Moreover, the artificial elevations of these nitrogenous substances can lead to severe pathophysiological conditions of the nervous system [e.g., (11)].

It is well known that uremia can also lead to perturbations in the thermoregulatory system. Prolonged uremia is often associated with hypothermia (9,10). Injecting urine or urea into experimental animals will result in a decrease in body temperature (2). It has been noted in patients with renal insufficiency that body temperature is inversely related to the degree of azotemia [i.e., blood levels of nitrogenous excretory substances; see (12)].

The thermoregulatory responses to uremia are comparable to those observed when rodents are treated acutely with various xenobiotic substances. For example, our laboratory has reported that compounds such as nickel, cadmium, sulfolane, and chlordimeform cause a reduction in body temperature and a preference for cooler ambient temperatures when administered intraperitoneally to mice (5,6). In view of the structural diversity of these compounds, the uniformity of the thermoregulatory response following acute exposure is somewhat surprising.

This leads to a hypothesis, "Are the thermoregulatory responses to the xenobiotic substances (heavy metals, etc.) driven by the same mechanisms as those responsible when animals become naturally toxemic such as would occur during

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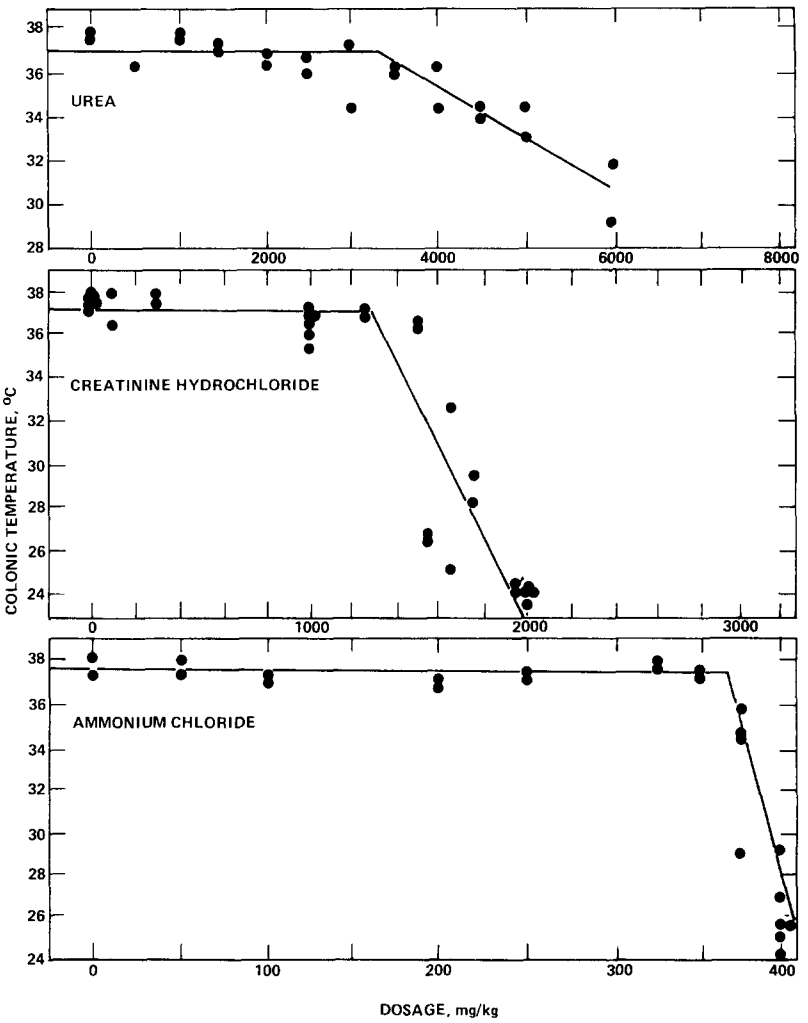

FIG. 1. Effect of increasing dosage of urea, creatinine, and ammonium chloride on colonic temperature in the mouse at an ambient temperature (T_a) of 20°C. Data fit with segmented linear regression functions (i.e., hockey-stick function).

the renal failure?" If the effects on behavioral and autonomic thermoregulation are similar then one could look for similar modes of action. On the other hand, if the thermoregulatory effects between nitrogenous and xenobiotic substances are dissimilar then one can argue for a unique thermoregulatory response for the xenobiotic substances. Hence, the purpose of this study was to assess the effects of acute toxic dosages of some key nitrogenous excretory products, namely, urea, creatinine, and ammonium chloride, on the body temperature and behavioral thermoregulatory response of the mouse.

METHOD

Animals used in this study were male mice of the BALB/c strain (Jackson Laboratories, Bar Harbor, ME). The animals were obtained at 30 days of age and were studied at an age of 8–10 weeks. The animals were housed in groups of 15 in cages lined with wood shavings and were maintained at an ambient temperature (T_a) of 22°C, a relative humidity of 50%, and a 12:12 L:D photoperiod. Food and water were provided ad lib.

Body Temperature

In an initial series of experiments a dosage response of the nitrogenous substances on body temperature was determined. Urea, creatinine (hydrochloride salt), and ammonium chloride (Sigma) were diluted in physiological saline to yield injection volumes of 0.1 ml per 10 g body mass.

A mouse was restrained momentarily and injected intraperitoneally (IP) with one of several dosages of one of the three nitrogenous substances. Dosages were selected on the basis of reported LD_{50} values in the literature (1) or from preliminary experiments. The injected mouse was placed in a ventilated stainless steel cage with dimensions of 7.7 (width) by 8.2 (length) by 9.6 (height) cm. The small cage was then placed in a larger environmental chamber thermostabilized to 20±1.0°C. One hour after injection the mouse was quickly removed from the environmental chamber and its colonic temperature was measured by inserting a thermocouple probe (Sensortek model RET-3) 17 mm beyond the anal sphincter for approximately 10 sec. The larger environmental chamber could accommodate six of the smaller stainless steel chambers. Hence, in a given experiment, six

mice could be tested simultaneously with a variety of dosages of the nitrogenous substances. The dosages were varied as the data were collected in order to construct a best-fitting dosage-response curve. The colonic temperature data were evaluated statistically using a segmented linear regression routine, also termed a "hockey-stick" function (8). Mice were administered only one injection of a given compound.

Behavioral Experiments

Once the dosage-response of each of the nitrogenous compounds on body temperature was determined, it was possible to select appropriate temperature-effective dosages to study behavioral thermoregulatory responses. Selected T_a's were determined in unrestrained mice by using a temperature gradient system [for details, see (4,5)]. Briefly, the system consisted of a square aluminum tube suspended between a hot and cold water bath. Inside the tube was a smaller ventilated tube into which a treated mouse was placed. Phototransistors along the length of the tube were used to monitor the position of the mouse in the temperature gradient. Position was converted to selected temperature on the floor of the gradient using a standard calibration curve of position versus temperature. Temperature in the gradient ranged from approximately 18 to 38°C.

A mouse was injected with one of the nitrogenous compounds and was then placed in the gradient for 90 min. Colonic temperature was determined immediately following removal from the gradient as described above. Selected temperature was calculated at two min intervals. Hence for each experimental run, there were 45 data points. These data points were reduced to nine sequential averages of selected temperature as a function of time in the gradient.

For each nitrogenous substance, a saline control, a low dosage which did not affect body temperature, and a high dosage which had a significant effect on body temperature were tested. Eight mice were tested at each dosage. The dosages selected were 0, 1000 and 4,500 mg/kg for urea; 0, 1000, and 1,700 for creatinine; and 0, 200, and 375 mg/kg for ammonium chloride. The mean selected temperature and colonic temperature in the gradient was statistically evaluated using Dunnett's multiple comparison t-test.

RESULTS

The response of colonic temperature to increasing levels of the nitrogenous substances displayed profound nonlinear characteristics (Fig. 1). Urea had no effect on body temperature up to a dosage of 3,280 mg/kg. Increasing the dosage above this level led to a decrease in colonic temperature (Fig. 1). The left- and right-hand confidence limits for the threshold dosage of urea were estimated as 1117 and 3868 mg/kg, respectively. The response to creatinine was much sharper compared to that of urea (Fig. 1). Colonic temperature was unaffected between dosages of 0 to 1279 mg/kg. Increasing the creatinine dosage above 1279 mg/kg led to an abrupt drop in colonic temperature. The left- and right-hand confidence limits for the threshold of creatinine were 1100 and 1407 mg/kg respectively. The colonic temperature response to ammonium chloride was qualitatively similar to that of creatinine in terms of the sharp break at a critical dosage (Fig. 1). The predicted threshold dosage was 365 mg/kg with left- and right-hand confidence limits of 358 and 370 mg/kg, respectively.

There appeared to be little effect of the nitrogenous substances on behavioral thermoregulation (Fig. 2). Control mice selected T_a's of 30.5 to 31.7°C. Urea at dosages of 1000 and 4500 mg/kg had no significant effect on the selected T_a. There was a slight but statistically significant decrease in the selected T_a of mice treated with a 1700 mg/kg dosage of creatinine (Dunnett's $t=2.86$, $p<0.05$). There was a small decrease in the selected T_a of mice given the highest dosage of ammonium chloride; however, the difference was not statistically significant. There was little variation in selected T_a with time.

Colonic temperature after 90 min in the temperature gradient was not significantly affected by any of the nitrogenous substances (Table 1). It should be noted that the highest dosages of creatinine and ammonium chloride were occasionally associated with what appeared to be small amounts of blood in the urine. Moreover, the highest dosages of these substances often led to severe ataxia and inactivity. All mice survived the highest dosages of the nitrogenous substances for at least the duration of the experiment.

DISCUSSION

The results of this study fail to support the hypothesis that the thermoregulatory responses following acute exposure to xenobiotic substances are similar to those observed when mice become toxemic following administration of high dosages of three principal nitrogenous substances. When the mice were placed in the temperature gradient after treatment with urea and ammonium chloride at dosages which cause significant hypothermia at a T_a of 20°C, the selected T_a did not differ substantially from that of the saline-treated mice. However, there was a slight decrease in the selected T_a at the highest dosage of creatinine.

The behavioral thermoregulatory responses following acute treatment with the nitrogenous substances were less impressive when compared to the large decreases in selected T_a noted in mice treated with xenobiotic substances. For example, mice treated with nonlethal dosages of sulfolane, an organic solvent, become hypometabolic, hypothermic and prefer relatively cool T_a's thereby behaviorally reinforcing the hypothermia. The treated mice could select relatively warmer T_a's and prevent the sulfolane-induced hypothermia (6). Mice treated IP with nickel and cadmium chloride prefer T_a's of approximatley 22°C and become severely hypothermic (5). Reducing body temperature following acute exposure to these xenobiotic agents may be beneficial to survival. There appears to be a direct correlation between xenobiotic-induced lethality and tissue temperature (6,7).

When given the option to behaviorally regulate their body temperature, mice given the nitrogenous substances at toxic dosages generally remain at relatively warm T_a's with a resultant insignificant effect on body temperature. However, it should be noted that a few individual animals given highest dosages of creatinine and ammonium chloride remained at the cool end of the temperature gradient and were severely hypothermic. These individuals accounted for the large variation in body temperature noted at these dosages. Mice given these high dosages were also very ataxic making it difficult to exclude an indirect effect via severe locomotive suppression.

It is clear that urine contains substances which evoke hypothermia in a variety of mammals, including man. Recently, Kluger and his colleagues determined that there is a proteinaceous substance in the urine of rabbit and man which promotes a regulated type of decrease in body temperature in the rabbit (2,9). A regulated decrease implies a reduction in the set-point for control of body temperature and is associated with physiological mechanisms that lower

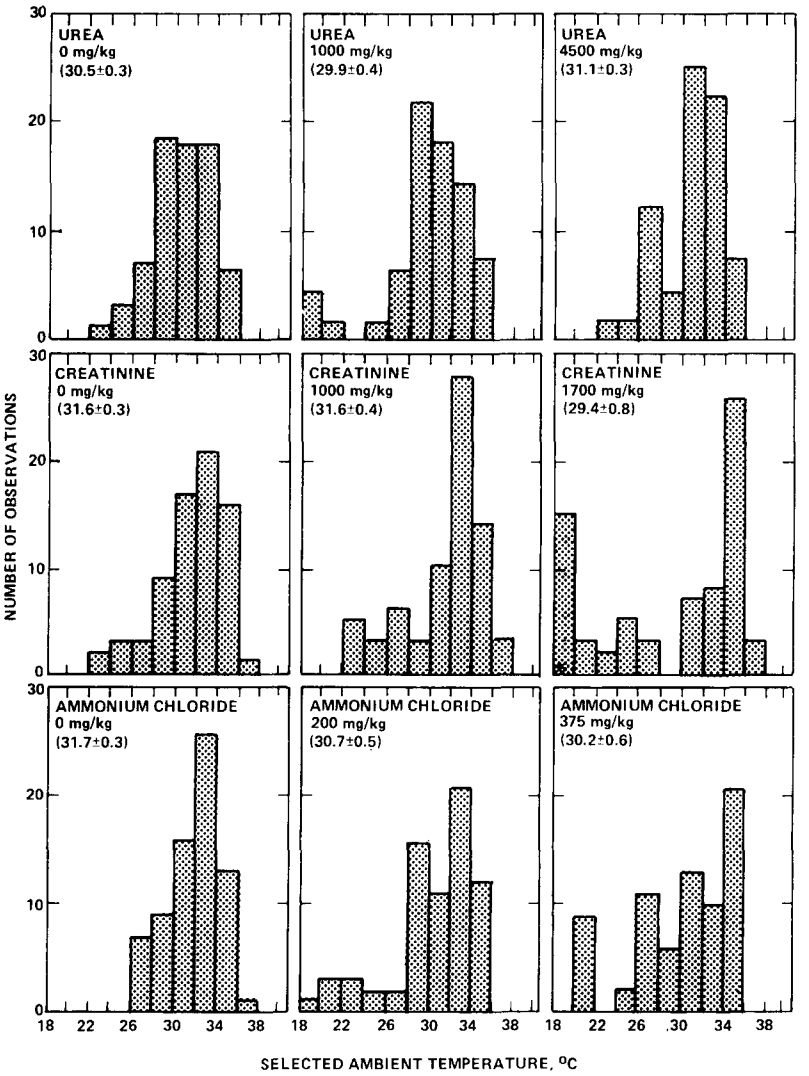

FIG. 2. Overall distribution of selected T_a in mice treated with various dosages of the nitrogenous compounds. The mean±S.E. of the selected T_a over the 90-min testing period is listed for each dosage.

TABLE 1

EFFECT OF UREA, CREATININE, OR AMMONIUM CHLORIDE INJECTION ON COLONIC TEMPERATURE OF MICE AFTER BEING PLACED IN THE TEMPERATURE GRADIENT FOR 90 MIN

Urea		Creatinine		Ammonium Chloride	
Dosage, mg/kg	Temperature, °C	Dosage, mg/kg	Temperature, °C	Dosage, mg/kg	Temperature, °C
0	36.5 ± 0.2	0	36.6 ± 0.12	0	36.7 ± 0.2
1,000	36.1 ± 0.2	1,000	37.1 ± 0.3	200	36.7 ± 0.3
4,500	35.6 ± 0.4	1,700	34.5 ± 2.0	375	35.0 ± 2.1

Data expressed as mean ± standard error. N=8 per dosage.

body temperature such as peripheral vasodilation, panting, and reduced shivering in cold environments. The proteinaceous substance responsible for the regulated hypothermia has been termed an "endogenous cryogen" (9). It was concluded that nonprotein substances in the urine did not play a major role in the regulated hypothermia because the urine's cryogenic properties could be deactivated by heating to a temperature of 97°C. There is no information on the effect of the urinary endogenous cryogen on behavioral thermoregulation; however, one would expect a reduction in the selected T_a following administration of the cryogen.

It seems clear that urea, creatinine, and ammonium chloride induce a forced and not a regulated hypothermia in the BALB/c mouse. Unfortunately, these substances had to be administered at relatively large dosages to evoke a thermoregulatory response. Hence, it is doubtful that further work using these substances would be beneficial. However, it is possible that other uremic toxins such as guanidines, uric acids, cyanate, pheols, and others (13) could evoke a regulated hypothermia similar to that observed when exposed to xenobiotic substances. Hence, it will be of future interest to determine if other classes of uremic toxins affect temperature regulation.

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