Circadian variation in the effects of nitric oxide synthase inhibitors on body temperature, feeding and activity in rats

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ORIGINAL ARTICLE

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Abstract We have investigated whether there is circadian variation in the effects of nitric oxide synthase inhibitors on body temperature, physical activity and feeding. We used nocturnally active Sprague-Dawley rats, housed at ≅24°C with a 12:12 h light:dark cycle (lights on 07:00 hours) and provided with food and water ad libitum. Nitric oxide synthesis was inhibited by intraperitoneal injection of the unspecific nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester (L-NAME, 100, 50, 25, 10 mg/kg), or the relatively selective inducible nitric oxide synthase inhibitor aminoguanidine (100, 50 mg/kg), during the day (≅09:00 hours) or night (≅21:00 hours). Body temperature and physical activity were measured using radiotelemetry, while food intake was calculated by weighing each animal's food before as well as 12 and 24 h after each injection. We found that daytime injection of L-NAME and aminoguanidine had no effect on daytime body temperature. However, daytime injection of both drugs did decrease nocturnal food intake ($P<0.05$) and activity ($P<0.05$). When injected at night, L-NAME reduced night-time body temperature ($P<0.01$), activity ($P<0.05$) and food intake ($P<0.05$) in a dose-dependent manner, but night-time injection of aminoguanidine inhibited only night-time activity ($P<0.05$). The effects of nitric oxide synthase inhibition on body temperature, feeding and activity therefore are primarily a consequence of inhibiting constitutively expressed nitric oxide synthase, and are subject to circadian variation.

Keywords Activity · Aminoguanidine · Body temperature · Circadian rhythm · Feeding · Nitric oxide · *N*-Nitro-L-arginine methyl ester

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Introduction

Inhibiting the synthesis of nitric oxide reduces evaporative water loss and skin blood flow [13, 15, 23], increases the rate at which body temperature rises during heat-exposure [17], and enhances the febrile response to endotoxin [18]. However, reducing nitric oxide synthesis also is reported to lower metabolic rate [15], cause hypothermia [34, 42], and decrease fever magnitude [21, 33, 34, 35]. The actions of nitric oxide in thermoregulation thus are ambiguous, with nitric oxide apparently stimulating both heat-loss and heat-gain mechanisms. In addition to its actions on thermoregulation, nitric oxide also influences physical activity and feeding behaviour, with decreased nitric oxide synthesis being associated with reduced physical activity [1, 6, 38] and food intake [7, 8, 10, 26, 27, 28].

Most studies investigating the role of nitric oxide in thermoregulation, physical activity and feeding have been performed on rodents, and all during the daytime. Yet, rodents exhibit strong circadian oscillations in body temperature (for review: [32]), feeding [46] and physical activity (for review: [32]), with the peak in each of these functions occurring at night. It occurred to us that some of the ambiguity regarding the thermoregulatory role of nitric oxide might arise because the experiments have been carried during the daytime, when body temperature is at its nadir, and thus when the full effects of manipulating nitric oxide concentration may not be evident. Similarly, the common use of exogenous stimuli such as starvation [7, 8, 10, 26, 27, 28] to increase daytime feeding, and stimulants [1, 6] or novel environments [38] to increase daytime physical activity, may have obscured the extent, or the timing, of nitric oxide's role in normal feeding and physical activity patterns. We therefore investigated the effects of manipulating nitric oxide concentration on daytime and night-time body temperature, food intake and physical activity levels in the absence of stressful exogenous thermoregulatory, physical activity and feeding drives. That is, we left the animals undisturbed during the study period, in a thermoneutral environment, with a 12:12 h light:dark cycle, and provided them with food and water ad libitum.

To manipulate nitric oxide, we used inhibitors of nitric oxide synthase, the enzyme responsible for the synthesis of nitric oxide from L-arginine. We used two inhibitors: one an unspecific inhibitor of all nitric oxide synthase isoforms, *N*-nitro-L-arginine-methyl ester (L-NAME), and the other a relatively selective inhibitor of inducible nitric oxide synthase, aminoguanidine. Thus we have attempted not only to ascertain the role of nitric oxide in normal feeding, physical activity and thermoregulation, but also to determine the relative contribution of nitric oxide produced by the constitutive and induced isoforms of the synthase enzyme.

Materials and methods

Animals

We used female Sprague-Dawley rats with a body mass of 200–250 g. The animals were housed individually in cages at a thermoneutral temperature of 24–25°C, and a light-dark cycle of 12:12 h (lights on at 07:00 hours). Food and water were provided ad libitum. All procedures were cleared by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2000/58/4).

Temperature measurement

Rats anaesthetized with 80 mg/kg ketamine and 20 mg/kg xylazine had sterile, wax-coated, temperature-sensitive radiotelemeters (Mini-Mitter, Sunriver, USA) implanted into their abdomens 7 days before the start of experiments. The telemeters were calibrated over a range of temperatures in a water bath, against a precision quartz-crystal thermometer (Quat 100, Heraeus, Germany), such that abdominal temperature could be measured to an accuracy of 0.1°C. The output frequency from each telemeter was monitored by a receiver plate (RTA 500, Mini-Mitter, USA) placed under each rat's cage. The frequency received by each plate was fed into a peripheral processor (Datacol-3 Automated Data Acquisition System, Mini-Mitter) connected to a personal computer, and the output expressed in degrees centigrade. Body temperature recordings were made at 10-min intervals.

Activity measurement

Detection of telemeter movement within the cage by the data-acquisition system described above allowed us to measure an index of each animal's activity. Activity was recorded as the accumulated activity count over a 10-min period.

Food intake

Food intake was determined by placing measured quantities of food, in excess of each rat's daily requirement, into the feed-trap of each cage at the time of injection, and then reweighing the food at the end of the experimental period. Food intake of animals injected during the day was measured over two 12-h periods (09:00–21:00 hours and 21:00–09:00 hours), while that of animals injected at night was measured only for the 12-h period following each injection (21:00–09:00 hours).

Drugs

L-NAME (Sigma, St Louis, Mo., USA) was dissolved in sterile pyrogen-free saline at a concentration of 100 mg/ml (L-NAME 100), 50 mg/ml (L-NAME 50), 25 mg/ml (L-NAME 25) or 10 mg/ml (L-NAME 10). Aminoguanidine (Sigma, USA) was also dissolved in sterile pyrogen-free saline at a concentration of 100 mg/ml (AG 100) and 50 mg/ml (AG 50). Fresh solutions were made up immediately before each injection.

Experimental procedure

Animals were each assigned to receive a single dose of one of the nitric oxide synthase inhibitors. Drugs were administered intraperitoneally (1 ml/kg) and each animal was injected with its drug and vehicle (sterile pyrogen-free saline) solution, on separate days, at 09:00 and 21:00 hours. Injections were spaced 7 days apart, and the time and order of the saline or drug injections were randomized.

Data analysis

All data are expressed as mean ±SD or mean ±SEM. For statistical purposes, abdominal temperature of each animal was consolidated into 2-h averages for the 24-h period following each injection. Two-way repeated-measures analysis of variance, followed by a Student-Newman-Keuls (SNK) post hoc test, was used to detect for differences within each group, differences between the vehicle and drug, and differences over time. In cases where analysis of variance detected significant interaction between main effects, interpretation of the data was based on the post hoc SNK test [16]. Relationships between drug dose, activity, food intake and temperature were described using regression and correlation analysis.

Results

Body temperature

Body temperatures of rats injected at 09:00 hours with nitric oxide synthase inhibitors or saline are shown in Fig. 1. Irrespective of whether saline, L-NAME or aminoguanidine was injected, a strong circadian rhythm of body temperature was maintained. Although L-NAME and aminoguanidine had no effect on the daytime body temperature of rats averaged over the full post-injection period (09:00–19:00 hours, Student's *t*-test, all groups: $P>0.05$), small, inconsistent, yet statistically significant deviations in body temperature were detectable on occasions, when L-NAME or aminoguanidine was administered (Fig. 1). Daytime injection of nitric oxide synthase inhibitors appeared to have no significant effect on night-time body temperatures, except when the highest dose of L-NAME was injected (Fig. 1: L-NAME 100). At that dose, body temperature was significantly lower, compared to that of saline-injected animals, between 03:00 and 05:00 hours on the morning following the injection (SNK, $P=0.03$). At the lowest dose of L-NAME (Fig. 1: L-NAME 10) and the higher dose of aminoguanidine (Fig. 1: AG 100), the onset of the body temperature rise at night appeared to be delayed.

Figure 2 shows the consequences for mean night-time body temperature of injecting rats at night (21:00 hours) with saline, L-NAME or aminoguanidine. Compared to the trivial effects that daytime injection of L-NAME had on body temperature, night-time injection of L-NAME caused a dose-dependent fall in nocturnal body tempera-

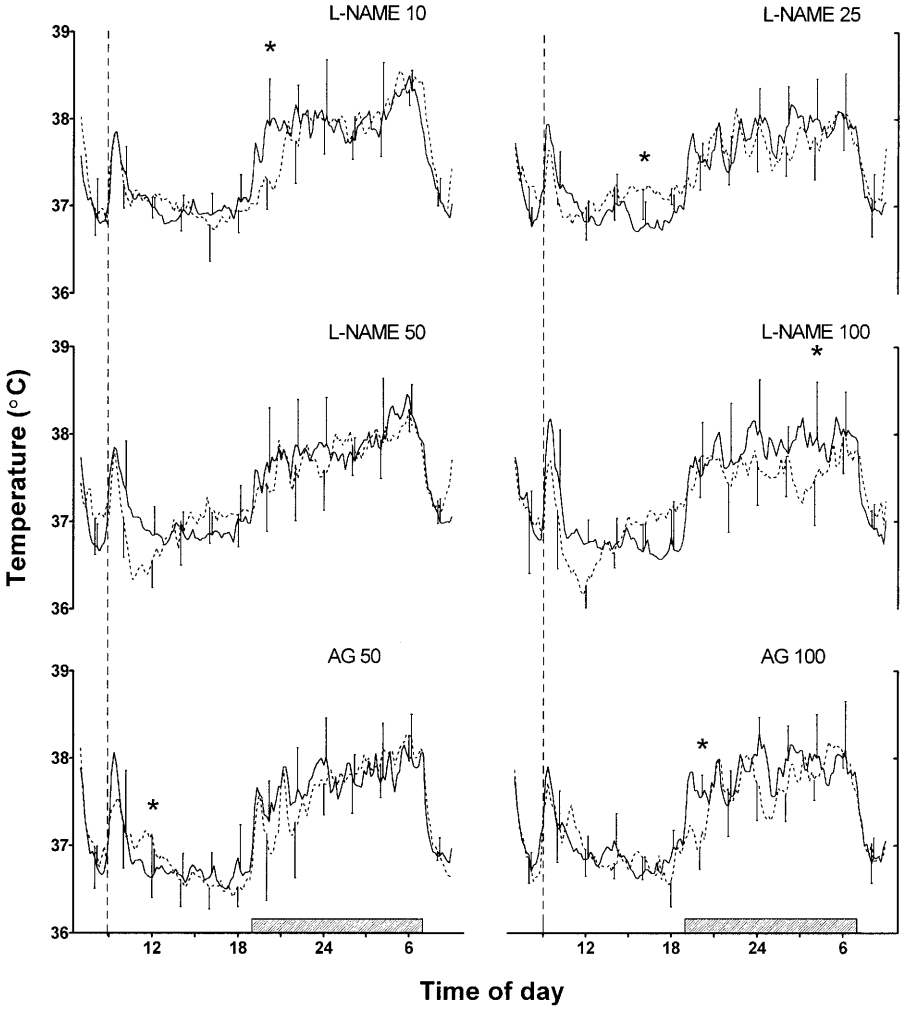

Fig. 1 Mean (±SD) 24-h body temperature of rats injected at 09:00 hours (*vertical dashed line*) with saline (*solid line*), *N*-nitro-L-arginine-methyl ester (*dashed line, L-NAME 10, 25, 50, 100* mg/kg) or aminoguanidine (*dashed line, AG 50, 100* mg/kg). Standard deviation bars are shown at 2-h intervals for clarity. *Shaded bars* indicate lights-off (19:00–07:00 hours). *$P<0.05$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

ture (linear regression, r^2=0.87, P=0.02, n=5), with the two highest doses of L-NAME causing hypothermia of ≅0.5°C (Fig. 2). The fall in body temperature caused by the injection of the two highest doses of L-NAME was not immediate, taking 1–2 h before the onset of hypothermia. Nevertheless, the mean post-injection night-time temperature (21:00–09:00 hours) of animals injected with 100 mg/kg L-NAME fell to temperatures similar to the mean daytime temperature (Student's *t*-test, P=0.21, Fig. 2: L-NAME 100). By 09:00 hours the next morning, mean body temperatures of all rats injected with L-NAME were not significantly different from those of rats injected with saline (SNK, all groups: P>0.05). Night-time injection of neither dose of aminoguanidine had an effect on nocturnal body temperatures (Fig. 2).

Food intake

The effect of morning (09:00 hours) injection of L-NAME or aminoguanidine on 24-h mean food intake is shown in Fig. 3. Mean food intake, in all animals, was lower over the daytime period (09:00–21:00 hours) than over the night-time period (21:00–09:00 hours), reflecting the well known circadian variation in feeding by rats. Injection of nitric oxide synthase inhibitors at 09:00 hours had little effect on daytime food intake, except for 50 mg/kg of L-NAME ($F_{(1,8)}$=14.844, P=0.003) and 100 mg/kg of aminoguanidine ($F_{(1,8)}$=19.003, P=0.002), which decreased daytime food intake (Fig. 3A, B). Interestingly, daytime injection of L-NAME or aminoguanidine affected night-time food consumption; both the 50 mg/kg (SNK, P=0.02) and the 100 mg/kg (SNK, P=0.02) doses of aminoguanidine decreased night-time food intake (Fig. 3D), while L-NAME inhibited nocturnal feeding in a dose-dependent manner (linear regression, r^2=0.8, P=0.02, n=5, Fig. 3C).

Mean food intake for the 12-h period after night-time (21:00 hours) injection of L-NAME or aminoguanidine is shown in Fig. 4. Night-time injection of aminoguanidine had no effect on nocturnal feeding (Fig. 4B). However, L-NAME injected at night caused a dose-dependent reduction in night-time food intake, as it did when it was injected during the day (linear regression, r^2=0.85, P=0.02, n=5, Fig. 4A).

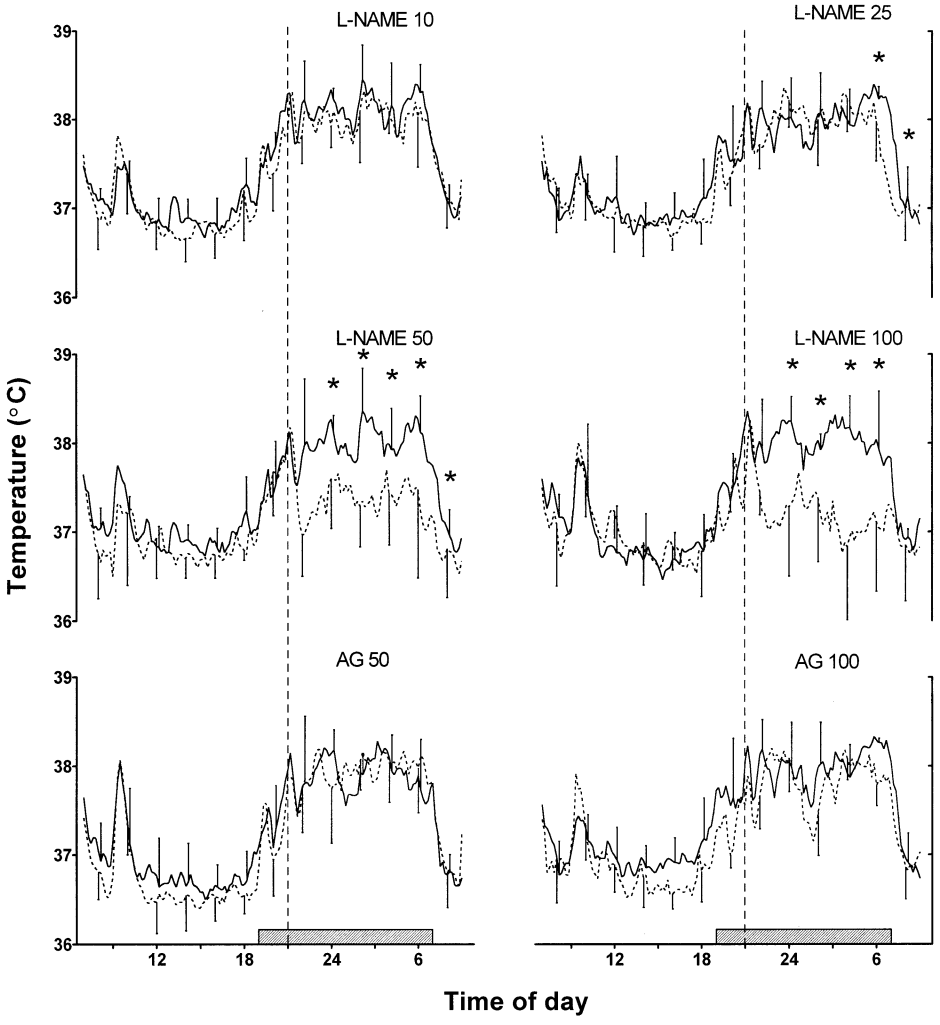

Fig. 2 Mean (±SD) 24-h body temperature of rats injected at 21:00 hours (*vertical dashed line*) with saline (*solid line*), *N*-nitro-L-arginine-methyl ester (*dashed line*, *L-NAME 10*, *25*, *50*, *100* mg/kg) or aminoguanidine (*dashed line*, *AG 50*, *100* mg/kg). Daytime temperatures preceding the injection are shown. Standard deviation bars are shown at 2-h intervals for clarity. *Shaded bars* indicate lights-off (19:00–07:00 hours). $^{*}P<0.01$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

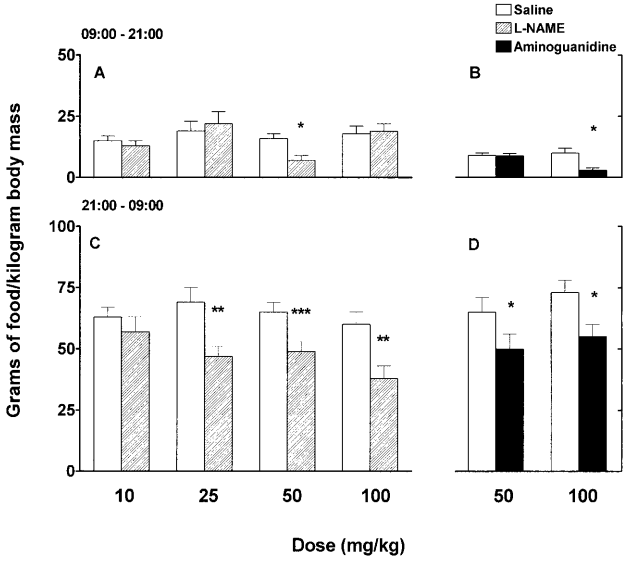

Fig. 3 Mean (±SEM) daytime (09:00–21:00 hours) and night-time (21:00–09:00 hours) food intake after daytime injection (09:00 hours) of saline or nitric oxide synthase inhibitors, L-NAME (**A**, **C**, doses: 10, 25, 50, 100 mg/kg) or aminoguanidine (**B**, **D**, doses: 50, 100 mg/kg). Lights-on 07:00–19:00 hours. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.01$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

Physical activity

As with body temperature and food intake, daily physical activity levels showed circadian variation, with highest activity levels occurring during the night. Figure 5 shows the mean activity of rats after they had been injected at 09:00 hours with nitric oxide synthase inhibitors or saline. Injection of L-NAME or aminoguanidine had no significant effect on the normally low daytime (09:00–19:00 hours) activity levels of rats (Fig. 5A, B). Nevertheless, daytime injection of both nitric oxide synthase inhibitors significantly reduced activity levels that night (SNK, all groups: $P<0.05$, Fig. 5C, D). No relationship between drug dose and the level of activity was found.

The effect of injecting L-NAME or aminoguanidine at 21:00 hours on night-time activity is shown in Fig. 6. Except for the lowest dose of L-NAME, nocturnal activity was significantly inhibited by the night-time injection of L-NAME (SNK, L-NAME 25, 50, 100 mg/kg: $P<0.05$, groups, Fig. 6A). Only the highest dose of aminoguanidine caused a night-time reduction in activity (SNK, $P=0.02$, Fig. 6B).

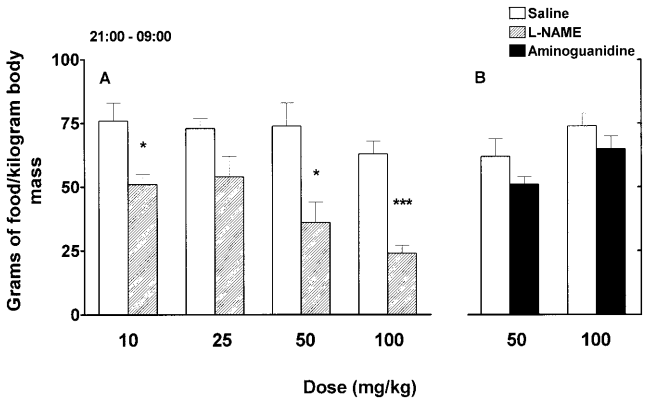

Fig. 4 Mean (±SEM) 12-h food intake (21:00–09:00 hours) after night-time injection (21:00 hours) of saline or nitric oxide synthase inhibitors, L-NAME (**A** doses: 10, 25, 50, 100 mg/kg) or aminoguanidine (**B** doses: 50, 100 mg/kg). Lights-off 19:00–07:00 hours. $^{*}P<0.05$, $^{***}P<0.01$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

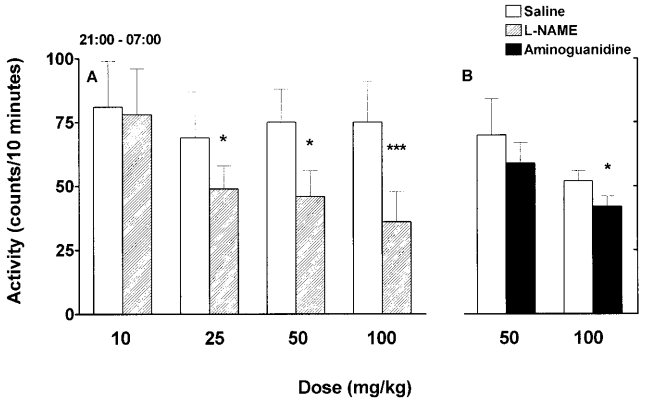

Fig. 6 Mean (±SEM) night-time (21:00–07:00 hours) activity after night-time injection (21:00 hours) of saline or nitric oxide synthase inhibitors, L-NAME (**A** doses 10, 25, 50, 100 mg/kg) or aminoguanidine (**B** doses 50, 100 mg/kg). Lights-off 19:00–07:00 hours. $^{*}P<0.05$, $^{**}P<0.01$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

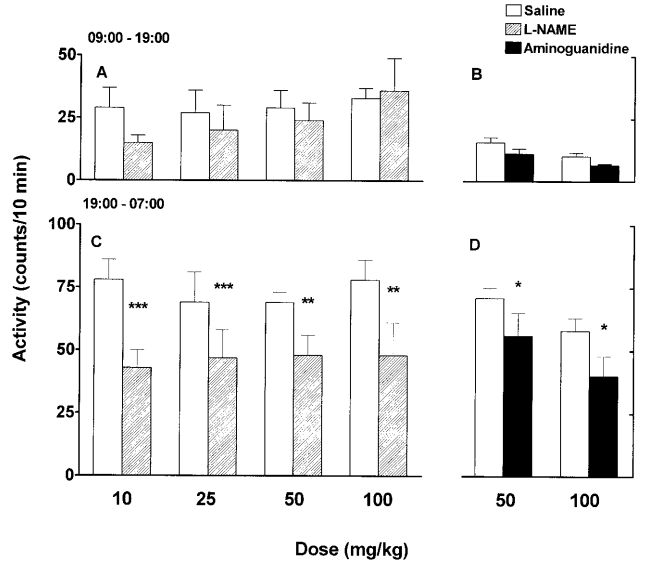

Fig. 5 Mean (±SEM) daytime (09:00–19:00 hours) and night-time (19:00–07:00 hours) activity after daytime injection (09:00 hours) of saline or nitric oxide synthase inhibitors, L-NAME (**A**, **C** doses 10, 25, 50, 100 mg/kg) or aminoguanidine (**B**, **D** doses 50, 100 mg/kg). Lights-on 07:00–19:00 hours. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.01$ versus saline (n=6: L-NAME 25, 100, AG 50; n=5: L-NAME 10, 50, AG 100)

Discussion

We have investigated the effects of inhibiting nitric oxide synthesis on body temperature, food intake and physical activity of rats at different stages of their sleep-wake cycle. We used two different nitric oxide synthase inhibitors: L-NAME, an unspecific inhibitor of all nitric oxide synthase isoforms, and aminoguanidine, a relatively specific inhibitor of inducible nitric oxide synthase. Two important findings emerge from our investigations. First, we are the first to show that there is strong circadian variation in the effects of inhibiting nitric oxide synthesis on body temperature, physical activity and feeding. While inhibition of nitric oxide synthesis had no major effects on daytime feeding, activity or body temperature, it did alter these three physiological functions at night. Our second important finding is the difference we uncovered between the efficacy of the unspecific nitric oxide synthase inhibitor, L-NAME, and the relatively specific inhibitor of inducible nitric oxide synthase, aminoguanidine, in modulating thermoregulation, feeding and physical activity. Whereas night-time injection of aminoguanidine decreased only nocturnal physical activity, night-time injection of L-NAME not only reduced night-time physical activity, but night-time food intake and body temperature too. The greater propensity for L-NAME to affect activity, feeding and thermoregulation indicates to us that nitric oxide synthesized by the constitutively expressed nitric oxide synthase enzymes is primarily responsible for nitric oxide's actions on these three physiological functions. We therefore have shown that there is significant circadian variation in the involvement of nitric oxide in physical activity, feeding and thermoregulation, and that this involvement probably depends on constitutively expressed nitric oxide.

In using L-NAME and aminoguanidine as tools to elucidate the effects of inhibiting nitric oxide synthase activity on thermoregulation, feeding and activity, we assumed that the drugs brought about their effects by direct inhibition of nitric oxide synthesis, and not as a consequence of other actions they may have had. Since we did not measure any markers of nitric oxide synthase activity, we cannot exclude other actions of the drugs as an explanation of the results we obtained. However, in addition to our drug doses being comparable to doses successfully used by other investigators to manipulate feeding, thermoregulation and physical activity [1, 7, 27, 33, 34, 38], there is in vivo

evidence that peripheral administration of L-NAME [19, 22, 24, 31], and of aminoguanidine [2, 20], at doses equal to or less than those doses used by us, decreases both peripheral and central nitric oxide synthesis. Moreover, a dose of aminoguanidine three times that used by us has been found to have no effect on other physiological variables; for example, blood pressure, arterial pH, blood glucose and blood gases [9]. In vitro studies, however, have shown that alkyl esters of L-arginine (e.g. L-NAME) are muscarinic receptor antagonists [4]. Even if L-NAME inhibited muscarinic receptors, especially at the higher doses we used, this action is unlikely to have had a major influence on the outcome of our study. Unlike the inhibition of nitric oxide synthase, the blockade of muscarinic receptors by L-NAME is not reversed by L-arginine [4]. Since the effects of nitric oxide synthase inhibition on feeding [7, 26, 27], activity [38] and body temperature (for review: [14]) are reversed by administration of L-arginine, it is unlikely that the effects L-NAME had on these three physiological functions are a consequence of muscarinic receptor blockade. Thus, we feel confident that decreased nitric oxide production is responsible for the effects the drugs had on body temperature, feeding and activity.

Nitric oxide has been ascribed both thermogenic and thermolytic functions, and thus its role in thermoregulation is ambiguous (for review: [14]). In afebrile animals, inhibition of nitric oxide synthesis by injection of L-NAME has been reported to cause hypothermia [34, 42], to have no effect on body temperature [15, 33], and to increase body temperature [23]. These differences in the effects L-NAME has on thermoregulation may be related to the route of injection (e.g. intravenous or intraperitoneal), the dose of drug used, the animal model used (e.g. rat, rabbit or guinea-pig), or whether or not the experiment was carried out in thermoneutral conditions. Like Roth and colleagues [33] we have found that decreasing the synthesis of nitric oxide with L-NAME has little or no effect on diurnal thermoregulation in rats (Fig. 1). Even though the two highest doses of daytime-injected L-NAME tended to cause small, transient decreases in body temperature, these changes in temperature were not statistically significant and were much smaller than the protracted night-time hypothermia caused by the same doses of L-NAME injected at night (Fig. 2). Thus, there is strong circadian variation in the effects of L-NAME on thermoregulation.

Inhibition of nitric oxide synthesis by L-NAME decreases activation of brown adipose tissue [10, 29]. However, there is no circadian variation in brown adipose tissue metabolism in rats provided with standard rat food [37], so it is unlikely that the circadian variation in the effects L-NAME has on thermoregulation are explained by its inhibitory actions on brown adipose tissue. Alternatively, the drop in body temperature may also have been the result of decreased nocturnal food intake [36], or decreased activity [40]. Although we found a significant positive linear correlation between night-time body temperature and food intake, when night-time food intake and activity were reduced by daytime injection of L-NAME or aminoguanidine (Figs. 3, 5), night-time body temperature was unaffected. In addition, other studies have shown that food deprivation affects daytime not night-time body temperature [45], and that the higher nocturnal body temperature of rats is not necessarily dependent on their higher night-time physical activity levels [32]. We believe therefore, that it was neither the decreased food intake nor the decreased activity that caused the L-NAME-dependent nocturnal decrease in body temperature.

Scales and Kluger [41] proposed that the increase in nocturnal body temperature in rats, in part, was a result of a prostaglandin-mediated increase in the temperature set-point. Nitric oxide reportedly increases cyclooxygenase activity [39], whilst decreasing the secretion of putative cryogenic agents (e.g. arginine vasopressin) by the hypothalamus [43]. Inhibition of nitric oxide synthesis, therefore, might increase the normally low night-time secretion of arginine vasopressin [44] and reduce prostaglandin synthesis, leading to a decrease in set-point and thus a decrease in nocturnal temperature. Arginine vasopressin, however, has been shown to not act as a cryogenic agent in female rats [30]. We believe, therefore, that reduced prostaglandin synthesis, rather than an increase in arginine vasopressin secretion, may have contributed to the night-time fall in body temperature of our rats.

Our findings that both daytime and night-time body temperatures are unaffected by aminoguanidine support observations already made by Roth and colleagues [35], who also found that aminoguanidine, as well as another inhibitor of inducible nitric oxide synthase inhibitor, *S*-methylisothiourea, had no effect on daytime body temperature in guinea-pigs. Thus, the view is developing that inhibitors of inducible nitric oxide synthase do not affect normal thermoregulatory function. This suggestion is not altogether surprising, since the inducible nature of the enzyme renders it unlikely to have a key role in the routine regulation of a tonically controlled physiological function such as body temperature. Conversely, inducible nitric oxide synthase has been shown to play a role in perturbations of thermoregulation, for example fever [21, 35].

Inhibition of nitric oxide synthesis causes a dose-dependent reduction of daytime food intake in chickens [7, 8], rats [10] and mice [26, 27, 28]. How exactly nitric oxide influences feeding behaviour is unknown, but it may involve the energy balance hormones, leptin and neuropeptide Y. Nitric oxide production in the hypothalamic feeding centres is reduced by leptin, an anorexigenic hormone [5, 28], and increased by neuropeptide Y, an orexigenic hormone [28]. Therefore, nitric oxide may function as a common messenger in the regulation of food intake, with the hypothalamic expression of nitric oxide being modulated by appetite-regulating hormones. Similarly, inhibition of nitric oxide synthesis also influences physical activity, reducing exploration of novel environments [38] and counteracting the stimulatory effects that morphine and methamphetamine have on activity [1, 6]. It has been proposed that nitric oxide affects locomotion by modulating the postsynaptic activity of neurons in the basal ganglia [1].

Contrary to the previous studies on rodents, neither daytime feeding (Fig. 3) nor locomotion (Fig. 5) in our ani-

mals was reduced by the daytime injection of nitric oxide synthase inhibitors, except for the small, though significant, reductions in daytime food intake we observed in animals injected with 50 mg/kg L-NAME or 100 mg/kg aminoguanidine (Fig. 3). We suggest, however, that these two isolated incidences were not drug-induced, but rather the result of random variation in each of those groups of animals. Indeed, in another group of rats, doubling the dose of L-NAME had no effect on food intake (Fig. 3A). The differences between our results and those of previous investigators may be methodological. Whereas other investigators induced daytime feeding by starving their animals beforehand [10, 26, 27, 28] and increased daytime physical activity levels through the use of stimulants [1, 6] or exposure to novel environments [38], our animals received food and water ad libitum and were left undisturbed, and thus had naturally low daytime food intake (Fig. 3) and activity levels (Fig. 5), against which no appreciable change during the daytime could be detected. In other words, in the absence of an artificially imposed drive on food intake or physical activity, the naturally low daytime food intake and activity levels of these nocturnal animals are not further reduced by inhibiting nitric oxide synthesis.

The results of our study, however, are congruent with the previously mentioned feeding and activity studies in so much that L-NAME did reduce food consumption and activity levels during periods of high food intake or activity, which in our case occurred naturally at night (Figs. 3, 4). This inhibition of night-time feeding and locomotion by L-NAME occurred irrespective of whether the drug was injected during the day (Fig. 3) or the night (Fig. 4). Since a single injection of L-NAME can inhibit the synthesis of nitric oxide for over 24 h [19], it is not surprising that the suppressive action of the drug on feeding and activity could be exerted long after its daytime injection, and at a time when food intake and activity is naturally elevated.

Our findings with respect to the effects of aminoguanidine injection on feeding and locomotion are more complex than those of L-NAME. That daytime injection of aminoguanidine, like L-NAME, could decrease night-time food consumption (Fig. 3) and activity (Fig. 5) is unexpected, since nitric oxide synthesized by the constitutively expressed nitric oxide synthase enzymes, not the inducible isoform, is thought to mediate decreases in food intake [5] and activity [11, 12]. Also, aminoguanidine is cleared from the plasma within hours of its injection [3], thus making a residual effect of the drug the night after a morning injection implausible. One possible explanation is that daytime injections of aminoguanidine disrupt daytime pathways that have subsequent effects on night-time feeding and activity, as illustrated by the ability of daytime but not night-time injections of aminoguanidine to affect night-time food intake. However, night-time injection of the highest dose of aminoguanidine did reduce night-time activity. Nevertheless, we believe that this effect of the highest dose of aminoguanidine was as a result of aminoguanidine inhibiting the constitutively expressed nitric oxide synthase, as it has been shown to do [25].

Rather than specific actions involving nitric oxide, generalized central nervous system depression, as a result of decreased nitric oxide synthesis [12], may be an alternative explanation for the nitric oxide synthase inhibitor-induced reductions in feeding, activity and body temperature. Dzoljic and colleagues [12] demonstrated a circadian rhythm in the depressive effects that nitric oxide synthase inhibitors have on the central nervous system, but the depressive actions peak during the day, out of phase with the reduced night-time feeding, activity and body activity we observed. Also, the normal daytime nadir of activity, food intake and body temperature would mask any depressant effects of nitric oxide synthase inhibition.

In summary, we have shown that the effects of nitric oxide synthase inhibition on body temperature, feeding and physical activity exhibit circadian variation, with stronger effects occurring at night, rather than by day, irrespective of the time of injection of the inhibitor. Daytime injections of L-NAME reduced night-time physical activity and feeding, while night-time injections of the drug decreased not only nocturnal feeding and activity but body temperature too, thus indicating that the control mechanisms of feeding and physical activity are more susceptible to changes in nitric oxide synthesis than those of body temperature. It is still unclear whether the circadian changes are as a result of rhythmic alterations in nitric oxide synthase gene expression, changes in the susceptibility of the enzymes to inhibitors, general central nervous system depression, or merely a consequence of low daytime body temperature, activity and food intake. However, our finding that daytime body temperature is refractory to inhibition of nitric oxide synthesis may provide some insight into the discrepancies that have been found in the apparent role nitric oxide plays in daytime thermoregulation. Also, we have shown that nitric oxide synthesized by the constitutively expressed nitric oxide synthase enzymes appears to have a role in normal thermoregulation, feeding and physical activity, but the role of nitric oxide synthesized by the inducible nitric oxide synthase in these physiological functions is unclear and requires further experimentation.

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