Pharmacology Biochemistry & Behavior, Vol 27, pp 431–435 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Effects of *d*-Amphetamine on Behavioral and Autonomic Thermoregulation in Mice[1]

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Received 5 February 1987

BUSHNELL, P J AND C J GORDON *Effects of* d-*amphetamine on behavioral and autonomic thermoregulation in mice* PHARMACOL BIOCHEM BEHAV 27(3) 431–435, 1987 —*d*-Amphetamine has well-known behavioral and sympathomimetic effects in rodents, but its effects on thermoregulation are not well characterized *d*-Amphetamine was administered IP to mice at doses of 0 1 to 10 0 mg/kg Locomotor activity and preferred ambient temperature (T_a) were measured for 60 min after injection in a linear temperature gradient, and metabolic rate (MR) and evaporative water loss (EWL) were measured in a metabolic chamber at ambient temperatures of either 20°C or 30°C Colonic temperatures (T_c) were obtained 60 min after injection in all cases Doses of *d*-amphetamine at 0 3 mg/kg and above reduced preferred T_a from the control value of 30°C to about 25°C Locomotor activity was reduced briefly by 0 3 mg/kg, and increased after 3 0 mg/kg *d*-amphetamine Metabolic rate was suppressed by 0 3 mg/kg of the drug at both 20 and 30°C At 20°C T_a, 10 0 mg/kg *d*-amphetamine increased MR but not EWL At 30°C, MR and EWL were both increased by doses of 3 0 and 10 0 mg/kg Body temperatures varied both as a function of *d*-amphetamine dose and of apparatus, with pronounced hyperthermia (T_c>38 5°C) evident only after 10 mg/kg in the metabolic chamber Thus, the behavioral and autonomic heat loss responses induced in mice by *d*-amphetamine suggest that its thermogenic action is detected by the animal at doses below those producing measurable thermogenesis and that appropriate effectors, from selection of a cool T_a to increasing EWL, are engaged in an orderly progression to maintain normothermia under all but the most challenging conditions

Thermoregulation *d*-Amphetamine Preferred ambient temperature Metabolic rate Evaporative water loss Body temperature Locomotor activity Mouse

THERMOREGULATION in mammals involves both behavioral and autonomic effectors The thermoregulatory system is highly sensitive to drugs [5] and other chemical agents [12–14] Despite the fact that an animal's behavioral repertoire contains a variety of energetically efficient thermoregulatory responses [10,21], behavioral thermoregulatory responses to intoxication have not been as thoroughly characterized as have autonomic responses Moreover, the interaction of behavioral and autonomic processes in response to intoxication has not been systematically evaluated

d-Amphetamine is a useful model compound for investigations of behavioral and autonomic processes involved in thermoregulation In an early study of behavioral thermoregulation, Weiss and Laties [23] showed that 1 to 2 mg/kg *d*-amphetamine caused rats to increase operant responding for heat reinforcement, while 4 mg/kg suppressed responding despite a drop in body temperature More recently, Yehuda and Wurtman [25] demonstrated that the colonic temperature (T_c) response of rats to high doses of *d*-amphetamine depended upon the ambient temperature (T_a) T_c was elevated in response to *d*-amphetamine at high T_a, and reduced at low T_a This *d*-amphetamine-induced hypothermia at low T_a has been examined in detail and appears to be mediated via central dopaminergic pathways [26,27] and to involve endogenous opioid peptides [24] At neutral ambient temperatures the effect of *d*-amphetamine on body temperature depends primarily upon dose high doses (>10 mg/kg) induced hyperthermia in rats [7], mice [16,17], rabbits [22], and cats [1], while low doses (ca 1 mg/kg) have been shown to induce hypothermia in unrestrained rats [15] and mice [18]

Thus *d*-amphetamine induces a variety of thermoregulatory responses in mammals depending upon T_a and dose (for review, see [5]) The means by which these responses are induced have not been clarified, though both autonomic and behavioral processs appear to be involved For example, metabolic rate in mice fell in response to low doses (≤10 mg/kg) of *d*-amphetamine given at 27°C [3] despite increased locomotor activity This effect, as well as *d*-amphetamine-induced hypothermia [18], appears to be centrally mediated, while the hyperthermia following larger doses appears to result from peripheral thermogenesis *per se* [8] Finally, very high doses appear to disrupt thermoregulation in rats completely rats given 15 mg/kg *d*-amphetamine in a cold environment avoided a heat lamp and became hypothermic, whereas in a warm environment they positioned themselves near a heat lamp and became hyperthermic [26]

An integrative study of both the behavioral and au-

[1]This manuscript has been reviewed by the Health Effects Research Laboratory, U S Environmental Protection Agency and approved for publication Mention of trade names or commerical products does not constitute endorsement or recommendation for use

tonomic responses of the rodent to *d*-amphetamine has not been made The purpose of this study was to evaluate interactions among behavioral and autonomic thermoregulatory responses to *d*-amphetamine by determining the effects of low doses of the drug on preferred T_a, locomotor activity, metabolic rate (as oxygen consumption), evaporative water loss, and T_c in the mouse

METHOD

Seventy-six adult male BALB/c mice, 6 to 8 weeks of age, were obtained from Jackson Laboratories (Bar Harbor, ME) and housed in groups of ten in cages lined with wood chips The housing room was maintained at a T_a of 22°C, relative humidity of 50%, and a 12 12 L D photoperiod with lights on at 0600 hr

Behavioral Measurements

Preferred T_a was measured using a linear temperature gradient previously described [9] Briefly, the gradient consisted of square aluminum tubing placed between hot and cold water baths An inner aluminum tube (5×5×76 cm) which contained the animal was positioned inside the larger tube Maximum and minimum floor temperatures in the tube ranged from 21 to 35°C Dry air was circulated from the warm to the cool end of the gradient at a flow rate of 500 ml/min Phototransistors placed at 2 5 cm intervals automatically monitored the position of the mouse in the gradient A standard calibration curve of position versus temperature was used to convert gradient position to preferred T_a A digital-analog recorder (Dianachart, Rockaway, NJ) printed the preferred T_a at two min intervals In addition, the relative change in position of the mouse in the gradient was measured to yield total longitudinal movement (i e , activity) in cm

d-Amphetamine sulfate (Sigma, St Louis, MO) was dissolved in physiological saline at concentrations of 0, 0 03, 0 09, 0 3, 0 9, and 3 0 mg of the base per ml, yielding dosages of 0, 0 1, 0 3, 1 0, 3 0, and 10 0 mg/kg with an injection volume of 0 10 ml/30 g body weight Each experimentally naive mouse (n=6/dose) was weighed, placed under mild restraint, injected IP with one of the six amphetamine solutions, and quickly placed in the thermally-equilibrated temperature gradient Preferred T_a was monitored at two-min intervals for 60 min The mouse was then removed from the gradient and its T_c measured by inserting a thermistor probe 2 5 cm past the anal sphincter T_c was not measured during the behavioral tests to minimize stress and to permit the animal complete freedom of movement in the gradient

Autonomic Measurements

Metabolic rate (MR) and evaporative water loss (EWL) were measured using an open-circuit indirect calorimeter as described previously [10] A temperature-controlled, airtight stainless steel chamber with a volume of 1 9 l contained a 13 cm diameter circular grid floor 6 5 cm below the top A layer of mineral oil beneath the grid floor served to prevent the moisture from urine and feces from interfering in the measurement of EWL Dry air was circulated through the chamber at a rate of 500 ml/min (STP) A fraction of the effluent air was pulled through a dew point hygrometer (General Eastern, Watertown, MA) to measure dew point temperature The air was dried and then pulled through an oxygen analyzer (Applied Electrochemistry, Sunnyvale, CA) to measure oxygen concentration The change in dew point temperature and oxygen concentration between the influent and effluent chamber air was used to calculate EWL and MR, respectively EWL was expressed as mg of water evaporated per ml of consumed oxygen MR was expressed as watts per kg (W/kg), assuming that 1 0 ml of consumed oxygen was equivalent to 20 1 J of heat [10]

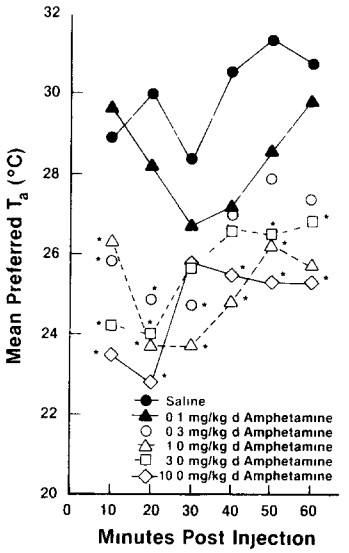

FIG 1 Mean preferred ambient temperature of mice in the temperature gradient as a function of *d*-amphetamine dose and time after injection Points with asterisks differ significantly ($p<0$ 05) from corresponding control (saline) points at each time interval

Experimentally naive mice were assigned randomly to one of 8 treatment groups (4 doses × 2 T_as, n=5/group) Then, following an IP injection as above of 0, 0 3, 1 0, or 10 0 mg/kg, each mouse was placed quickly inside the environmental chamber maintained at a T_a of either 20 or 30°C Oxygen concentration and dew point temperature of the effluent chamber air were recorded at two-min intervals Sixty min after *d*-amphetamine injection, each mouse was quickly removed from the chamber and its colonic temperature measured as described above

Statistical Analysis

The recordings of preferred T_a, activity, MR, and EWL were sequentially averaged into 10 min blocks Thus, for each 60 min experiment, there were six observations of preferred T_a and activity for the behavioral study and six measurements of EWL and MR for the autonomic study

Preferred T_a and locomotor activity were analyzed by 2-way analyses of variance (ANOVAs *d*-amphetamine dose by time) with repeated measures for time Metabolic rate and EWL were analyzed by 3-way (dose by T_a by time) ANOVAs, again with time as a repeated measure (General Linear Model [20]) Significant 3-way interactions were partitioned into separate *d*-amphetamine dose by time analyses at each T_a, in which the effect of *d*-amphetamine was examined at each time point using Dunnett's procedure for comparing experimental means with a control [19] T_c was analyzed in the temperature gradient by a one-way ANOVA on *d*-amphetamine dose, while T_c in the metabolic chamber required a dose by T_a analysis Data from one mouse each in the 0 3 mg/kg and 10 0 mg/kg groups in the

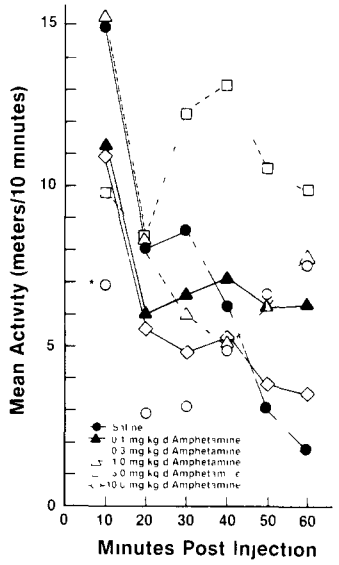

FIG 2 Mean locomotor activity of mice in the temperature gradient as a function of *d*-amphetamine dose and time after injection Asterisks as in Fig 1

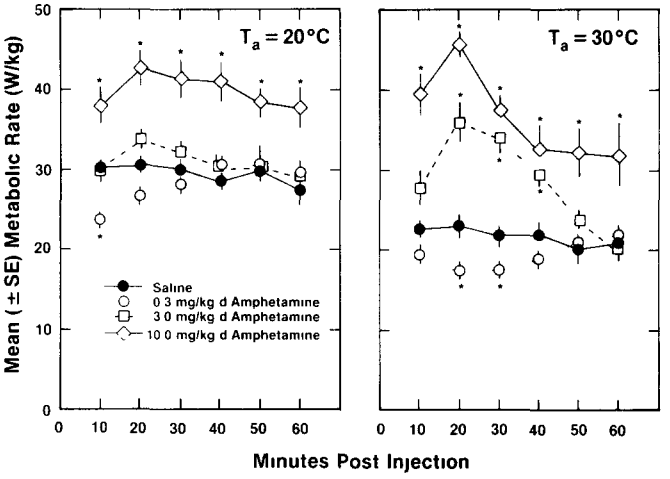

FIG 3 Mean (±S E) metabolic rate of mice in the metabolic chamber at ambient temperatures of 20°C (left) and 30°C (right) as a function of *d*-amphetamine dose and time after injection Asterisks as in Fig 1

behavioral experiment were discarded as outliers [6] The criterion for statistical significance was 0 05 for all tests

RESULTS

Preferred T_a

Preferred T_a in the temperature gradient was reduced initially by *d*-amphetamine in a dose-related manner, and returned toward control values over time (Fig 1) Preferred T_a was significantly affected by all *d*-amphetamine doses above 0 1 mg/kg [*d*-amphetamine main effect, $F(5,29)=4\ 07$, $p<0\ 0064$, *d*-amphetamine by time interaction, $F(25,145)=1\ 94$, $p<0\ 0133$] Preferred T_a recovered to control values in the 0 3 mg/kg *d*-amphetamine group by the end of the test period, while the preferred T_a of mice given higher doses did not (Fig 1)

Locomotor Activity

Locomotor activity in the temperature gradient (Fig 2) declined over time blocks differentially across *d*-amphetamine treatments [*d*-amphetamine by time interaction, $F(25,145)=3\ 18$, $p<0\ 0004$] *d*-amphetamine at 0 3 mg/kg significantly reduced locomotor activity in the first 10 min of the test, locomotor activity was significantly increased above control 50–60 min after 3 0 mg/kg *d*-amphetamine (Fig 2)

Metabolic Rate

MR (Fig 3) was higher at 20°C than at 30°C after all doses of *d*-amphetamine, $F(1,32)=43\ 15$, $p<0\ 0001$ In addition, *d*-amphetamine significantly increased MR, $F(3,32)=59\ 98$, $p<0\ 0001$, overall, with a more pronounced effect at 30°C than at 20°C [*d*-amphetamine by T_a interaction, $F(3,32)=3\ 60$, $p<0\ 024$] Significant changes in the effect of *d*-amphetamine over time were also observed at both T_as, 0 3 mg/kg reduced MR for 10–30 min, and 10 0 mg/kg increased it at all times after treatment *d*-Amphetamine at 3 0 mg/kg did not affect MR at 20°C, but elevated it at 30°C from 20 to 40 min postinjection

Evaporative Water Loss

EWL was not significantly affected by *d*-amphetamine at 20°C T_a, $F(3,16)=2\ 34$, NS, but was significantly elevated by all doses of *d*-amphetamine at 30°C T_a, $F(3,16)=62\ 56$, $p<0\ 0001$ (Fig 4) At 30°C T_a, analysis of the significant *d*-amphetamine by time interaction, $F(15,80)=5\ 53$, $p<0\ 0001$, showed that all doses of the drug elevated EWL for the first 10 min, while from 20–60 min postinjection, only 3 0 and 10 0 mg/kg *d*-amphetamine increased it (Fig 4)

Colonic Temperature

T_c following the injection of saline was lower in the gradient than in the metabolic chambers (Table 1) T_c was significantly increased in the gradient by 0 3, 1 0, and 10 0 mg/kg *d*-amphetamine, $F(5,28)=4\ 15$, $p<0\ 01$ In the metabolic chamber, however, T_cs were significantly reduced by 3 0 mg/kg *d*-amphetamine and increased by 10 mg/kg *d*-amphetamine (Table 1) These effects were independent of T_a [main effect of *d*-amphetamine $F(3,39)=11\ 92$, $p<0\ 0001$, main effect of T_a $F(1,32)=2\ 63$, NS, interaction $F(3,32)=1\ 28$, NS] Since the interaction was not significant, statistical evaluations in Table 1 refer to the main effect of *d*-amphetamine and the asterisks denote significant changes in T_c as a function of dose only

DISCUSSION

d-Amphetamine affected both behavioral and autonomic thermoregulatory responses in a dose-dependent manner The highest (10 0 mg/kg) dose of *d*-amphetamine significantly elevated MR (Fig 3) and T_c (Table 1) at both 20°C and 30°C After 3 mg/kg, *d*-amphetamine increased MR at 30°C, but not at 20°C These doses induced clear attempts to lose

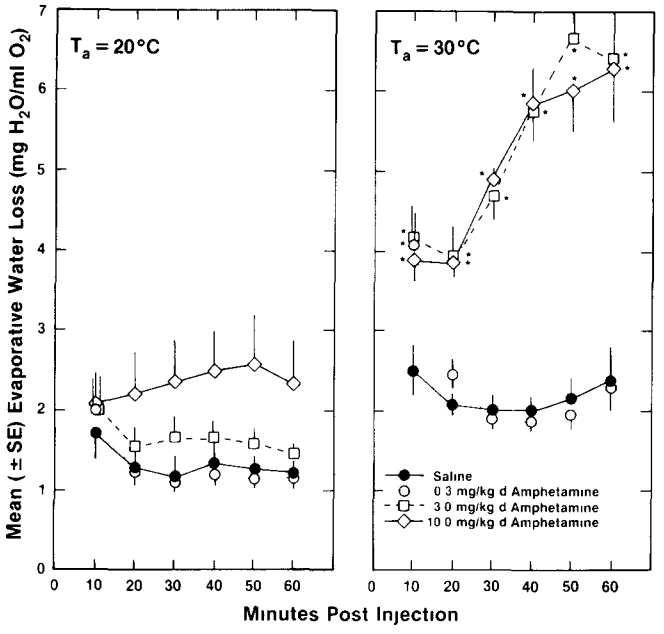

FIG 4 Mean (±S E) evaporative water loss of mice in the metabolic chamber at ambient temperatures of 20°C (left) and 30°C (right) as a function of *d*-amphetamine dose and time after injection Asterisks as in Fig 1

heat, including a preference for reduced T_a (Fig 1) and an increase in EWL (Fig 4) These heat loss responses were sufficient to prevent hyperthermia after 3 mg/kg, but not after 10 mg/kg *d*-amphetamine, in fact, 3 mg/kg *d*-amphetamine caused slight but significant hypothermia in the metabolic chamber (Table 1)

These behavioral and autonomic responses suggest that the 10 0 mg/kg dose of *d*-amphetamine caused a forced elevation of body temperature, in which appropriate heat loss effectors (i e , selection of a cooler T_a and increase in EWL) were activated to compensate for the thermogenic effect of the chemical [9] There was no clear dose-response relationship between dose of amphetamine and T_c in the behavioral or metabolic experiments The T_c of the control group in the metabolic chamber was unusually high at a T_a of 20°C However, in spite of the unusual responses at the lower doses, it is apparent that the highest dose of *d*-amphetamine caused significant hyperthermia in mice placed in the temperature gradient and metabolic chamber Because of the small size of the mouse, the colonic temperature measurement can occasionally be labile, this may explain the unusual dose-effect functions observed

It is notable that the two higher doses of *d*-amphetamine increased MR to approximately the same absolute levels, independent of T_a This increase in MR contrasts with that of the control mice, which varied predictably with T_a (Fig 3), and probably reflect peripheral thermogenic effects of the drug, not under CNS control, as previously reported [8] By contrast, the lowest dose used in the autonomic experiment, 0 3 mg/kg, suppressed MR to roughly the same amount *relative* to control rate at both T_as (Fig 3) This latter observation is consistent with centrally-mediated suppression of CO_2 production induced by low doses of *d*-amphetamine [3], and suggests that this MR suppression may be part of an integrated, CNS-mediated homeothermic response to low doses of *d*-amphetamine

TABLE 1

EFFECTS OF *d*-AMPHETAMINE ON T_c (°C ± SEM) IN THE PREFERRED T_a GRADIENT AND IN THE METABOLIC CHAMBER AT BOTH T_aS

d-Amphetamine Dose (mg/kg)	Gradient	Metabolic Chamber 20°C		30°C
0	36 8 ± 0 28	38 0 ± 0 13		37 7 ± 0 24
0 1	37 3 ± 0 26	—		—
0 3	38 0 ± 0 10*	37 7 ± 0 20		38 1 ± 0 10
1 0	37 7 ± 0 14*	—		—
3 0	37 2 ± 0 20	36 8 ± 0 29	*	37 3 ± 0 33
10 0	37 7 ± 0 19*	38 5 ± 0 35	*	39 4 ± 0 61

Asterisks indicate significant departures from respective control means in the gradient and metabolic chamber In the metabolic chamber, the statistical comparisons are made without regard for T_a (see the Results section)

In addition to suppressing MR for 10 min, 0 3 mg/kg *d*-amphetamine also reduced preferred T_a for 10–30 min and locomotor activity for 10 min, and increased EWL (for 10 min at 30°C T_a) (Figs 1–3) This pattern of results suggests that this brief suppression of MR was part of a constellation of heat loss responses triggered by low doses of *d*-amphetamine This pattern further suggests that thermal receptors within or outside the CNS are extremely sensitive to *d*-amphetamine and elicit corrective responses in the absence of an amphetamine-induced change in thermogenesis and body temperature This is supported by previous work reporting hypothermic effects of small intraventricular injections of *d*-amphetamine [2,18]

The reduction of locomotor activity by 0 3 mg/kg *d*-amphetamine during the first 10 minutes after treatment in the preferred T_a gradient (Fig 2) is unusual, and may be related to the unique physical characteristics of this apparatus (i e , temperature extremes at either end of a long, narrow aluminum tube) The expected increase in activity was observed 50 to 60 min after 3 0 mg/kg of the drug, however

It thus appears that *d*-amphetamine induces a series of heat loss responses in mice which change both in degree and kind with increasing dose First, the lowest effective dose of the drug (0 3 mg/kg) caused the mouse to select a low T_a (Fig 1) and suppress its MR (Fig 3) As dose increased, the preferred T_a remained low, at 3 0 to 10 0 mg/kg (depending upon T_a Fig 3), MR increased as the peripheral thermogenic effects of the drug [8] overcame its centrally-mediated tendency to suppress MR [3] and T_c [18] After the highest *d*-amphetamine doses and at high T_a, EWL increased to counteract the peripheral thermogenic effects of the drug (Fig 4) This integrated pattern of response, beginning with selection of a low T_a and culminating with increased EWL, maintained the mouse close to normothermia at all except the most severe combination of pharmacological and environmental challenges This pattern contrasts to that of the rat, which at high dosages of *d*-amphetamine at least, displays a multiphasic response without clear relationship to changes in body temperature and behavior [25–27] The responses of the rat to similar challenges, at dose and T_a levels which do not break down thermoregulation altogether, would be of great interest to pursue

ACKNOWLEDGEMENTS

The able technical assistance of M D Long and R A Workman is greatly appreciated We thank L Cook, R Janssen, and C Liddle for their reviews of the manuscript

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