

Influence of thyroid hormones on 3,4-methylenedioxymethamphetamine-induced thermogenesis and reinforcing strength in monkeys

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In monkeys, elevating ambient temperature has been shown to increase sensitivity to 3,4-methylenedioxymethamphetamine (MDMA) reinforcement. Earlier rodent studies have shown that elevations in thyroid levels (hyperthyroidism) parallel changes in elevating the ambient temperature on MDMA-induced thermogenesis, but the interaction has not been examined in monkeys. This study was designed to evaluate the effects of chronic levothyroxine (3.0 or 4.5 µg/kg/day, intramuscularly; Levo) treatment on MDMA-induced increases in body temperature following 1.5 mg/kg (intravenously) MDMA and self-administration when MDMA (0.03–0.3 mg/kg/injection) and food were available under a concurrent fixed-ratio 30 schedule of reinforcement in rhesus monkeys ($n=4$). Earlier studies had shown that 1.5 mg/kg MDMA did not affect thermoregulation at 24°C. Chronic Levo treatment resulted in significant increases in MDMA-induced thermogenesis. In the self-administration experiment, MDMA choice increased with dose, such that food was preferred over saline and a low MDMA dose (0.03 mg/kg/injection), whereas 0.1 and 0.3 mg/kg/injection MDMA was preferred over food. Although elevating ambient temperature had been shown to

increase MDMA potency, there was no effect of chronic Levo treatment on MDMA choice. These results suggest that changes in thyroxine levels do not parallel the changes in ambient temperature in altering the reinforcing strength of MDMA. *Behavioural Pharmacology* 19:167–170 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA)-induced thermogenesis and reinforcement are influenced by ambient temperature in both rodents and nonhuman primates (Gordon *et al.*, 1991; Cornish *et al.*, 2003; Banks *et al.*, 2007, 2008; Von Huben *et al.*, 2007). MDMA-induced thermogenesis in rodents is also thyroid hormone dependent (Sprague *et al.*, 2003, 2007). Clinically, there is a case report suggesting that hyperthyroidism potentiated the hyperthermic effects of ecstasy (Martin *et al.*, 2007). Currently, there is a void in our understanding of the effects of thyroid hormones on MDMA-induced thermogenesis and reinforcement in monkeys. Previously, we reported that elevating the ambient temperature produced changes in MDMA-induced thermoregulation and increased the potency of MDMA reinforcement (Banks *et al.*, 2007, 2008). In this study, we hypothesized that elevating thyroid hormone levels would have similar effects as elevating the ambient temperature on MDMA-induced thermogenesis and the relative reinforcing strength of MDMA under a concurrent fixed-ratio (FR) schedule of MDMA and food choice. An advantage of

concurrent schedules of reinforcement is that the dependent variable, drug choice, is less influenced by the direct effects of the drug on response rates and that response allocation is related to changes in reinforcement magnitude (Katz, 1990; Woolverton and Nader, 1990; Bergman and Paronis, 2006).

Methods

Subjects

Four individually housed adult male rhesus monkeys (*Macaca mulatta*), surgically prepared with an indwelling intravenous catheter and a subcutaneous vascular access port (Access Technologies, Skokie, Illinois, USA; Martelle *et al.*, 2007), served as subjects. All monkeys were previously trained under a concurrent FR 30 schedule of food and MDMA (0.03–0.3 mg/kg/injection) presentation (Banks *et al.*, 2008). Monkeys were weighed biweekly and fed enough food daily (LabDiet #5038 Monkey Chow and fresh fruit) to maintain body weights at approximately 95% of free-feeding levels. Rectal temperatures were taken at least three times per week after Levo administration but before the behavioral

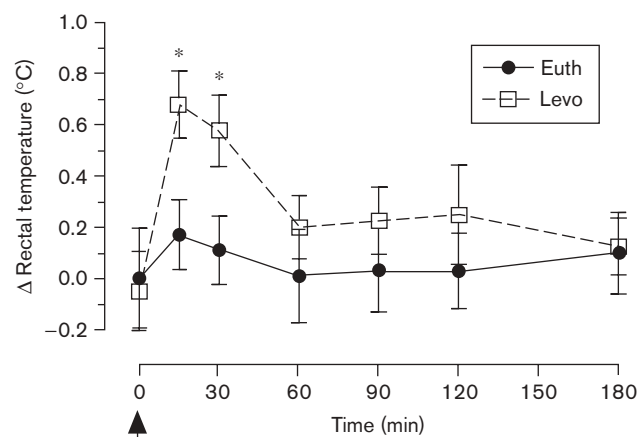
session. The number of food pellets earned was calculated into the daily food requirements and monkeys were fed at least 2 h after completion of the experimental sessions. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University (WFU) Nonhuman Primate Environmental Enrichment Plan. All procedures were approved by the Animal Care and Use Committee of WFU and performed in accordance with the 2003 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

Procedure

Experimental sessions were conducted in ventilated and sound-attenuating chambers (Med Associates, East Fairfield, Vermont, USA) with the ambient temperature inside the chamber maintained at $24 \pm 1^\circ\text{C}$ as described previously (Banks *et al.*, 2008). Briefly, the skin above the venous access port was cleaned with betadine and 95% ethanol, and the port was connected to the infusion pump via a 22-gauge Huber point needle. At the beginning of each session, the pump was operated for approximately 3 s to fill the port and catheter with the concentration of MDMA or saline available for that session. Sessions began with illumination of lights above both levers. Thirty consecutive responses on a lever resulted in either a 10-s infusion or delivery of a 1-g banana-flavored pellet, followed by a 10-s timeout. The levers designated as food and drug were randomly determined for each monkey and remained constant throughout the experiment. Sessions ended after 30 total reinforcers had been earned or 60 min had elapsed. Each dose was available for at least five consecutive sessions and remained available until responding was deemed stable (percent injection-lever responding $\pm 20\%$ of the mean of three consecutive sessions with no trend). Doses were tested in mixed order in each monkey.

After completion of the MDMA dose–response curve, the effects of Levo were examined. At the start of Levo ($3.0 \mu\text{g/kg/day}$, intramuscularly) treatment and for 10 consecutive days, responding was only maintained by food presentation under an FR 30 schedule of reinforcement before reinstituting MDMA self-administration. This dose of Levo was based on pilot data demonstrating behavioral effect as determined by a shift in the MDMA dose–effect curve. Levo treatment continued when the MDMA self-administration dose–response curve was redetermined after 10 days of Levo treatment. In two monkeys in which the dose–effect curve was not altered after the $3.0 \mu\text{g/kg/day}$ Levo treatment, the Levo dose was increased to $4.5 \mu\text{g/kg/day}$ for 10 days, and the MDMA dose–effect curve redetermined. During Levo treatment, all behavioral sessions began approximately 60 min after levothyroxine administration.

Fig. 1



Effects of chronic levothyroxine treatment (Levo; open symbols) on MDMA (1.5 mg/kg , intravenously)-induced changes in rectal temperature. Data (mean $\pm$ SEM, $n = 4$) are represented as change ($^\circ\text{C}$) from baseline ($38.4 \pm 0.2^\circ\text{C}$; time 0 min) temperature. Asterisks indicate significantly different from baseline ($P < 0.05$). Closed symbols (Euth; baseline thyroid levels) represent effects of MDMA at room temperature (from Banks *et al.*, 2007). Arrow indicates time of MDMA administration. MDMA, 3,4-methylenedioxymethamphetamine.

At the conclusion of the behavioral experiments, monkeys were challenged with noncontingent MDMA (1.5 mg/kg , intravenously) approximately 24 h after the last Levo injection to assess the effects of chronic Levo treatment on MDMA-induced thermogenesis. Rectal temperature was recorded for 3 h using a RET-1 rectal probe attached to a TH-3 thermocouple (Physitemp; Clifton, New Jersey, USA). For comparison, the effects of 1.5 mg/kg MDMA on thermogenesis in the absence of Levo treatment (Banks *et al.*, 2007) are included in Fig. 1 and designated as euthyroid (Euth).

Drugs

Levothyroxine sodium (Bedford Laboratories, Bedford, Ohio, USA) was obtained from the WFU Baptist pharmacy and dissolved in sterile saline. ($\pm$)MDMA HCl was provided by the National Institute on Drug Abuse (Bethesda, Maryland, USA) and dissolved in sterile saline.

Plasma thyroxine levels assessment

Before and 10 days after initiating Levo treatment, venous blood (2 ml) was collected and transferred to a Vacutainer tube with no additives (Becton-Dickinson, Franklin Lakes, New Jersey, USA). Blood samples were centrifuged at 3000 rpm for 10 min at 4°C , and thyroxine levels were determined by Antech Diagnostics (Lake Success, New York, USA).

Data analysis

Plasma thyroxine levels at baseline and after Levo were analyzed using a paired *t*-test. Temperature data were

analyzed using a one-way ANOVA with time as the factor followed by a Dunnett post-hoc test comparing each time point to baseline (time 0). Behavioral data were analyzed with repeated measures mixed linear regression using SAS Proc Mixed (Version 8.2, SAS, Cary, North Carolina, USA) with Levo, MDMA dose and their interaction as factors. For the self-administration experiments, the primary dependent variables examined were MDMA choice (calculated by dividing the total number of MDMA-lever responses by the total responses on both levers), number of MDMA and food reinforcers earned per session. Differences were considered significant at the 95% level of confidence ($P < 0.05$).

Results

Effects of chronic Levo treatment on plasma thyroxine levels

Chronic Levo treatment significantly increased ($t = 4.99$, $P < 0.05$) plasma thyroxine levels on average 160% from baseline ($6.3 \pm 0.6 \mu\text{g/dl}$; $n = 4$) in the absence of MDMA.

Effects of chronic Levo treatment on 3,4-methylenedioxymethamphetamine-induced changes in temperature

Chronic Levo treatment did not significantly change basal rectal temperature measured over the 3-month treatment period ($38.3 \pm 0.1^\circ\text{C}$ vs. $38.4 \pm 0.2^\circ\text{C}$). Administration of noncontingent ($\pm$) MDMA (1.5 mg/kg, intravenously) at 24°C significantly increased rectal temperature [$F(6,27) = 3.16$, $P < 0.05$; Fig. 1]. Post-hoc analysis revealed a significant ($P < 0.05$) change in rectal temperature at 15 and 30-min post-MDMA that returned to baseline at 60 min.

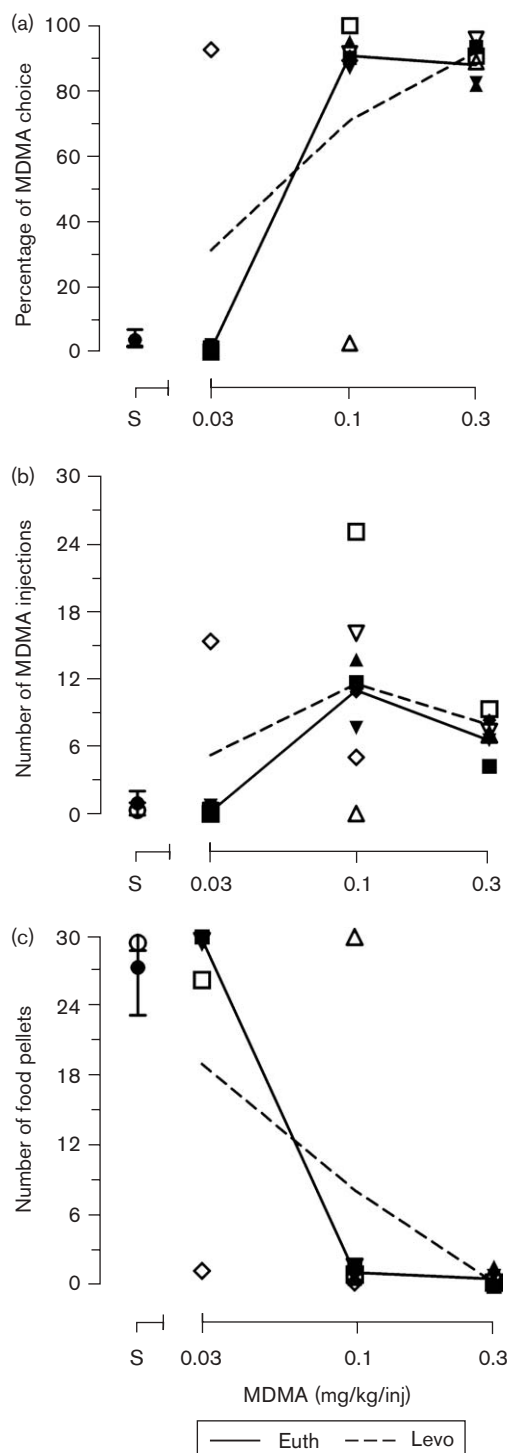
Effects of chronic Levo treatment on 3,4-methylenedioxymethamphetamine choice

Under baseline conditions, when the choice was between saline or a low dose of MDMA (0.03 mg/kg/injection) and food, monkeys primarily chose food (Fig. 2a; filled symbols). Increases in MDMA dose resulted in increases in MDMA choice, such that 0.1 and 0.3 mg/kg/injection MDMA were primarily ($P < 0.05$) chosen over food. A significant main effect of MDMA dose was present for MDMA choice [$F(3,9) = 26.85$, $P < 0.05$; Fig. 2a], number of MDMA injections earned [$F(3,9) = 6.81$, $P < 0.05$; Fig. 2b] and number of food pellets earned [$F(3,9) = 30.7$, $P < 0.05$; Fig. 2c]. Chronic Levo treatment did not significantly alter any dependent measure.

Discussion

The rationale for these studies was based on the parallel effects of elevating the ambient temperature or thyroid hormone levels on potentiating MDMA-induced thermogenesis (Gordon *et al.*, 1991; Sprague *et al.*, 2003, 2007). We had previously shown that noncontingent MDMA

Fig. 2



Effects of chronic levothyroxine (Levo) treatment on MDMA choice (a), MDMA injections (b) and food pellets (c) under a concurrent schedule of MDMA and food availability. Abscissa represents MDMA dose (0.03–0.3 mg/kg/injection). Data (mean $\pm$ SEM, $n = 4$) above S represent when saline was the alternative to food. Individual monkeys are represented by different symbols with filled symbols indicating baseline thyroid levels (Euth) and open symbols indicating chronic levothyroxine (Levo) condition. The solid or dashed lines indicate the group means at Euth or during Levo treatment, respectively.

administration did not significantly change rectal temperature at 24°C (Banks *et al.*, 2007). In this study, after chronic Levo treatment, MDMA administration at 24°C did significantly increase rectal temperature. Although the topography of the thermogenic effect was different (peak thermogenic effect was 15 min) compared with elevating the ambient temperature under identical conditions (peak thermogenic effect was 90 min; Banks *et al.*, 2007), this finding confirms a parallel effect of elevating the ambient temperature and increasing thyroid hormone levels on MDMA-induced thermogenesis and extends the role of thyroid hormones in MDMA-induced thermogenesis to monkeys.

Preclinical research has demonstrated an interaction between ambient temperature and MDMA reinforcement (Cornish *et al.*, 2003; Banks *et al.*, 2008). For example, elevating ambient temperature increased low-dose MDMA choice in the same monkeys used in this study under identical schedule conditions (Banks *et al.*, 2008). In contrast to the effects of elevating the ambient temperature, elevating thyroxine levels did not increase the relative reinforcing strength of MDMA despite having an effect on MDMA-induced thermogenesis. Individual differences were noted, unrelated to Levo dose. These findings support the contention that elevating thyroxine levels and elevating ambient temperature, which have parallel effects on MDMA-induced thermoregulation, do not have similar consequences on MDMA reinforcement. The effects of ambient temperature on reinforcement appear not to be mediated through thermoregulatory mechanisms, such as the hypothalamic-pituitary-thyroid axis.

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