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Effects of Cold Acclimation and Central Opioid Processes on Thermoregulation in Rats

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WILSON, J. R. AND B. A. HOWARD. *Effects of cold acclimation and central opioid processes on thermoregulation in rats.* PHARMACOL BIOCHEM BEHAV **54**(2) 317-325, 1996.—Two experiments, using centrally administered [D-Ala^2-MePhe^4-Gly(ol)^5]enkephalin (DAMGO), a selective μ-opioid agonist, assessed the thermoregulatory consequences of cold acclimation. Experiment 1 assessed whether cold acclimation influenced DAMGO hyperthermia at room temperature. Sialoadenectomized rats were implanted with ICV cannulae and IP Mini-Mitters. After 3 weeks of exposure to 5°C (cold acclimation) or 22°C (non-cold acclimation) rats were pretreated with IP naltrexone HCl (2 mg/kg b.wt.) or vehicle (0.15 M saline) and later administered a 5-μl ICV injection of 0.15 M saline, 0.1, or 1.0 μg DAMGO. Cold acclimation exerted little effect on core temperature but potentiated DAMGO hyperthermia in a dose-dependent, naltrexone-reversible, activity-independent manner. Experiment 2 assessed the effect these same manipulations exerted on operant escape from a convective source of mild heat (37°C). Duration of heat escape increased with cold acclimation in a naltrexone-resistant manner, yet was not influenced by DAMGO in either non-cold-acclimated or cold-acclimated rats. These findings suggest that two central adaptations occur with cold acclimation: A non-μ-opioid process that increases heat sensitivity and a μ-opioid process that potentiates hyperthermia but fails to alter heat escape due to μ-opioid-mediated analgesia.

Cold acclimation DAMGO Core temperature Heat escape Telethermistors Rats

PROLONGED cold exposure improves cold tolerance in small mammals (39,40). This improvement occurs in the context of several adjustments, including elevations in obligatory and regulatory nonshivering thermogenesis (8,23), increases in sympathetic innervation of brown adipose tissue (11,21), enhanced metabolic reactivity to certain diets or exogenously administered catecholamines (23,36), and elevations in plasma and tissue (25,55) catecholamines. In a laboratory setting such adjustments constitute cold acclimation.

Cold acclimation may also influence the thermoregulatory actions of central opioids. In rats, low doses of centrally injected morphine, a phenanthrene alkaloid and prototypic opioid receptor ligand, generally produce hyperthermia (1). Similarly, centrally administered β-endorphin, an endogenous opioid peptide and *C*-terminal fragment of β-lipotropin that binds predominantly with μ-opioid receptors (35), elevates body temperature (6) and plasma catecholamines (49,50). However, despite the negligible effects of short-term (24-h) cold exposure (22), the metabolic (53) actions of intraventricular injections of β-endorphin are enhanced in anesthetized rats after 3 weeks of cold acclimation. The paucity of μ-selective ligands and the tendency for shorter peptides to degrade rapidly in vivo (35) may have discouraged an assessment of μ-opioid receptors' participation in this cold acclimation-induced metabolic hyperreactivity. However, [D-Ala^2-MePhe^4-Gly(ol)^5]enkephalin (DAMGO) offers a biologically stable, synthetic opioid peptide with over 200- and 3000-fold higher affinity for μ- than for δ- and κ-opioid receptor binding sites, respectively (19,41). In conscious non-cold-acclimated rats, centrally injected DAMGO, like both morphine and β-endorphin, produces hyperthermia (44,51) and increases sympathetic nerve activity and plasma catecholamines (29,30, 42). These thermal and neurohormonal effects of both β-endorphin and DAMGO are generally antagonized with opiate antagonists and ganglionic blockers. Accordingly, Experiment 1 was designed to assess, in a neutral ambient temperature,

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whether cold acclimation potentiates the hyperthermic action of ICV injections of DAMGO in a dose-dependent, naltrexone-reversible manner.

Cold-acclimated rats tend to substitute exercise-induced heat loads for heat normally generated by shivering and nonshivering thermogenesis (3,20). Whether heat generated from spontaneous motor activity could substitute in a similar manner is unclear, but the facts remain that heat generated from motor activity influences body temperature, and that both cold exposure (31) and low doses of morphine or β-endorphin (4,14) increase motor activity. The potential, therefore, for motor activity to contribute secondarily to DAMGO hyperthermia led to the decision to monitor motor activity. In a similar regard, β-endorphin and dynorphin reportedly induce a naloxone-reversible increase in grooming (2,15). This consideration, coupled with the tendency for grooming to offset hyperthermia through facilitating water evaporation (18), and thus constituting a potential buffer to DAMGO hyperthermia, led to the use of sialoadenectomized rats.

EXPERIMENT 1

METHOD

Subjects

Twenty-eight adult male, Sprague–Dawley rats obtained from the University of Manitoba's Central Animal Care Service were initially housed in individual wire mesh hanging cages with free access to Wayne Rat Blox and water. The colony room was maintained on a 12 L : 12 D cycle (lights on 0800–2000 h) at 22 ± 2°C and 40% relative humidity, within the optimal thermoneutral range recommended for housing rats (31,54).

Apparatus

Core temperature and skeletal motor activity testing arena. The effect of DAMGO on skeletal motor activity and core temperature was assessed in a 90 × 90-cm plywood box, painted white, with 60-cm high walls around the borders. A 3-mm-thick removable Plexiglas sheet with a grid system of 10 × 10-cm squares covered the floor of the box. A radio antenna was placed beneath the box to receive pulses from thermal transmitters chronically implanted in the rats and the entire apparatus was housed in a room maintained at 22°C.

Telemetric monitoring of core temperature. A 2.3-g AM transmitter (19 × 12 mm, Model VM; Mini Mitter Inc., Sunriver, OR) was implanted into the peritoneal cavity of each rat. The transmitters emitted AM pulses at rates that are linearly proportional to core temperatures. Before implantation, each transmitter was calibrated by establishing its pulse rate while it was immersed in a controlled temperature, circulating water bath (Lauda-Model B-1) at five temperatures ranging from 30 to 40°C. The telemetry pulses were captured by an AM receiver and then passed through an electronic circuit that amplified and recorded the pulses on an IBM personal computer.

Surgical Procedures

For all surgical procedures, animals were anaesthetized with sodium pentobarbitol (60 mg/kg, IP, Allen and Hansbury) supplemented with 0.2 ml atropine sulfate (0.4 mg/ml, IP) to suppress mucosal secretions. The site of the incision was shaved, and the skin was wiped successively with an alcohol swab and sterile saline. All incisions were sutured with 00 silk surgical suture, and a topical antibacterial cream was applied to the incision (Furacin, 0.2%, Austin). Then a local analgesic (2% Xylocaine HCl Astra) and an intramuscular injection of a broad spectrum antibiotic (Ethacilin, Rogar STB, 45,000 units) were administered.

Unilateral ICV cannulation. Following the induction of anaesthesia, all rats had their heads shaved and then placed in a Kopf stereotaxic instrument. The cannula was a modified tuberculin syringe. The syringe tip and corresponding needle were threaded firmly together, and a 24-ga guide tube was mounted inside the tip with acrylic cement to a depth of 5 mm. The tip of the guide tube was implanted with the coordinates, 0.8 mm posterior, 4 mm ventral, and 1.5 mm lateral to the left side of bregma, to allow injections into the lateral ventricle. A 26-ga injector cannula extended 1.0 mm below the guide cannula. The cannula was anchored with three stainless steel jeweller's screws and dental acrylic cement. An inner stylet was positioned in the guide cannula until the day of the experiment.

Sialoadenectomy. Following ICV cannulation, all animals were desalivated to minimize access to competing modes of heat loss using a previously described procedure (9,52), which involved making bilateral incisions from each ear to an imaginary midventral line extending from the hyoid bone caudal to the manubrium. The underlying parotid, submaxillary, and major sublingual glands were separated from connective tissues, arteries to these glands were ligated, and the glands removed. The incisions were sutured with 00 surgical silk and a local analgesic was applied.

AM transmitter implantation. To implant a Mini-Mitter capsule into the peritoneal cavity, a 2–3-cm incision was made on the lateral surface of the abdomen, immediately in front of the hind legs. An aseptic Mini-Mitter was then inserted into the peritoneal cavity and sutured to the peritoneal wall.

Drugs. The drugs DAMGO (Sigma, 0.1 and 1.0 μg) and naltrexone HCl (Sigma) were prepared separately. Naltrexone (*N*-cyclopropylmethyl-7,8-dihydro-14-hydroxynormorphine HCl or EN-1639A) was selected for use as a narcotic antagonist that binds to a high-affinity μ-opioid receptor site (32). It is nearly twice as potent, and longer acting, on a milligram basis than naloxone, but has fewer agonistic properties and untoward side effects than cyclazocine or other benzomorphan derivatives (5,16,33). The desired amount of each drug was weighed on a Mettler balance (Model AE 166) and then dissolved in 0.15 M sterile saline. Fresh drug solutions were made daily, and the solutions' pHs were monitored. The volume for all ICV injections was 5 μl. Injection into the lateral ventricles was verified by postmortem visual inspection for perfused India ink.

Procedure

Pretest. On day 1, half the rats (n = 14) were assigned to the cold-acclimated (CA) condition and the remainder (n = 14) were assigned to the non-cold-acclimated (NCA) condition. Animals assigned to the CA conditions were housed in a walk-in cooler (Coldstream Products of Canada, Model WIDC) that was maintained at 5 ± 2°C and kept on a 12 L : 12 D cycle (lights on at 0800 h) for 26 consecutive days. These animals had separate polypropylene cages with wood shavings for bedding and free access to food and water. Animals assigned to the NCA conditions were housed in a colony room maintained at 22 ± 2°C with similar housing conditions, including lighting, cages, bedding, and access to food and water.

On day 7, all subjects underwent aseptic surgery for im-

plantation of cannulae and Mini-Mitters and for sialoadenectomy. Following a 48-h postoperative recovery at 22°C, each animal was returned to its home cage, at either 5 or 22°C. On day 22, a 2-day apparatus adaptation procedure began that involved placing a rat in the open field for 1 h each day. This procedure was intended to minimize the potential thermal consequences of an otherwise novel environment on either core temperature or skeletal motor activity observed by other investigators (42).

Drug testing. The CA and NCA animals were assigned to either a VEH-DAMGO group, or a NLX-DAMGO group. Subjects in the VEH-DAMGO (n = 14) group received 0.15 M saline (1 ml kg^{-1}, IP) injections followed 40 min later by ICV injections of either saline or one of two doses of DAMGO. Subjects in the NLX-DAMGO (n = 14) group received a naltrexone (2 mg kg^{-1}, IP) injection followed 40 min later by an ICV injection of either 0.15 M saline or one of the two doses of DAMGO. Accordingly, this experiment consisted of four groups, each having seven subjects: CA-VEH-DAMGO, NCA-VEH-DAMGO, CA-NLX-DAMGO, and NCA-NLX-DAMGO. Within each group, rats were exposed to each DAMGO dose (0.15 M saline, 0.1, and 1.0 μg/5.0 μl) in a counterbalanced order.

Core temperature response and skeletal motor activity to DAMGO were tested on days 24–26. Twelve hours prior to each testing session, both NCA and CA subjects were relocated into a separate temperature equilibration room (22 ± 2°C). The testing procedure involved placing each subject into the open field for a 15-min readaptation. An ICV injection of either saline or naltrexone was administered by inserting a 26-ga stylette attached to a 10-μl syringe (Hamilton, Reno, NV) through the guide cannula while holding the rat. The rat was then replaced into the center of the open field. For the following 40 min, of which the last 30 min constituted baseline, the number of squares traversed in the open field and core temperature was recorded every 10 min. A traverse was defined as the movement of the rat from one square to a second square on the grid flooring using the shoulder blades as reference points. Each rat was then removed from the arena, administered a 5-μl ICV injection of either saline or one of two doses of DAMGO, and replaced into the center of the arena. The number of squares traversed and core temperatures were again recorded at 10-min intervals during an 80-min test session. After testing, the rat was removed from the open field and replaced in its home cage. The open field was then cleaned with a dilute acetic acid solution. This procedure was repeated on days 25 and 26, such that by the end of the day 26 test session each rat had been tested on each of the possible drug combinations for its group.

Statistical Analyses

Baseline values for body weight, core temperature, and activity were analyzed in separate 2 × 2 (Acclimation Condition × Drug Pretreatment) factorial, 2 × 2 × 3 × 3 (Acclimation Condition × Drug Pretreatment × Test Day × Sampling Time) mixed factorial, and 2 × 2 × 3 (Acclimation Condition × Drug Pretreatment × Test Day) mixed factorial analyses of variance (ANOVAs), respectively. Post-DAMGO values for changes in core temperature from baseline and changes in activity from mean baseline activity obtained after DAMGO administration were analyzed in 2 × 2 × 3 × 8 (Acclimation Condition × Drug Pretreatment × DAMGO Dose × Sampling Time) mixed factorial ANOVAs with repeated measures on DAMGO Dose and Sampling Time, whereas 2 × 2 × 3 (Acclimation Condition × Drug Pretreatment × DAMGO Dose) mixed factorial ANOVAs with Dose as a repeated measure summarized changes in mean baseline activity and peak (maximum) changes from mean baseline core temperature. The ANOVAs were followed by trend analyses for selected main effects and interactions. The alpha level for all analyses was set at $p < 0.05$.

RESULTS

Baseline Values

Body weight. Pretest body weights did not differ (Fs < 1) with either acclimation or drug pretreatment manipulations (mean = 213.2, SE = 2.4 g).

Core temperature. Cold-acclimated (CA) rats possessed slightly higher core temperatures (mean = 37.3, SE = 0.1°C) than NCA rats (mean = 36.9, SE = 0.1°C), $F(1, 24) = 4.74$, $p < 0.05$. No other main effects or interactions were obtained for Pretreatment, Test Day, or Sampling Time (Fs < 1).

Activity. Activity diminished linearly, $F(1, 24) = 7.23$, $p < 0.01$, across the three 10-min sampling intervals for all animals from a high of mean = 114 squares traversed/10 min at baseline onset to mean = 85 squares traversed during the last 10 min. No additional main effects or interactions attributable to either Acclimation condition, Drug Pretreatment, or Test Day were obtained (Fs < 1).

Post-DAMGO Values

Core temperatures (fig. 1). Across all pretreatment conditions the elevation from baseline in core temperature progressively increased during the 80-min test period, $F(7, 168) = 6.61$, $p < 0.001$. This dose-dependent hyperthermia, $F(2, 48) = 38.0$, $p < 0.001$, was lowest for the saline dose (mean = 0.0, SE = 0.04°C) and highest for the 1.0-μg dose of DAMGO (mean = 1.0, SE = 0.02°C). However, the Drug Pretreatment effect, $F(1, 24) = 4.81$, $p < 0.05$, indicated that across all doses the DAMGO hyperthermia obtained in the vehicle-pretreated rats (mean = 0.71, SE = 0.03°C) was suppressed by naltrexone (mean = 0.39, SE = 0.03°C). The Drug Pretreatment × Sampling Interval, $F(7, 168) = 4.43$, $p < 0.001$, and Drug Pretreatment × DAMGO Dose, $F(2, 48) = 14.8$, $p < 0.001$, interactions revealed that the temperature differences developed progressively over the test session and that the hyperthermia obtained at 0.1 μg and 1.0 μg DAMGO for vehicle-pretreated rats (mean = 0.8, SE = 0.03°C and mean = 1.4, SE = 0.03°C, respectively) exceeded that observed in naltrexone-pretreated controls (mean = 0.4, SE = 0.03°C and mean = 0.6, SE = 0.02°C, respectively).

For the NCA rats trend analyses exhibited a Dose × Pretreatment interaction, $F(2, 24) = 5.23$, $p < 0.01$, indicating that the body temperatures for the naltrexone-pretreated rats (mean = 0.7°C) were lower than those of the vehicle-pretreated rats (mean = 1.2°C) for the highest DAMGO dose. Moreover, the hyperthermia in the vehicle-pretreated controls exhibited a marginal quadratic profile over the testing session, $F(1, 6) = 5.25$, $p < 0.06$. In CA rats DAMGO elicited an overall linear dose-dependent hyperthermia, $F(2, 24) = 18.15$, $p < 0.001$. However, the Dose × Pretreatment interaction, $F(2, 24) = 9.80$, $p < 0.001$, indicated that the elevated body temperatures of naltrexone-pretreated rats at the 0.1-μg (mean = 0.3°C) and 1.0-μg (mean = 0.5°C) doses were lower than those of vehicle-pretreated rats (means = 1.0 and 1.7°C, respectively). For the vehicle-pretreated CA rats,

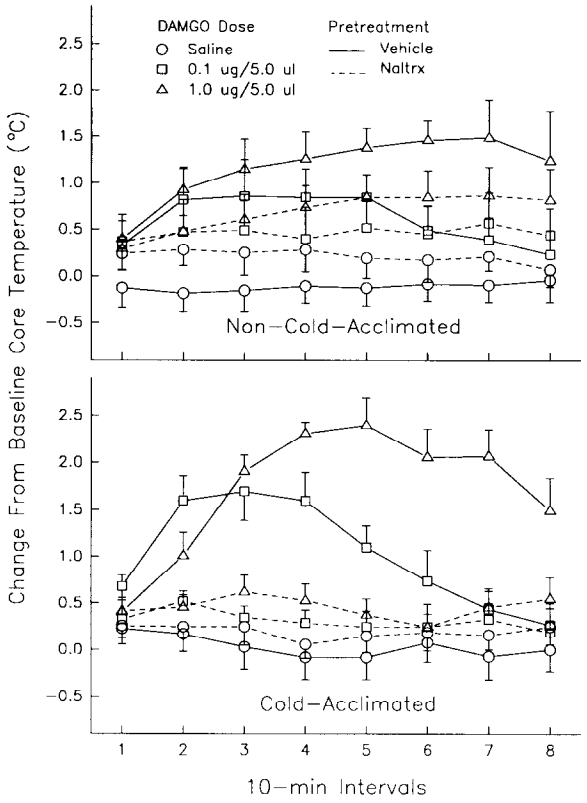

FIG. 1. Mean ± SEM change from baseline core temperature in naltrexone- and vehicle-pretreated non-cold-acclimated and cold-acclimated rats within 80 min of ICV administration of one of three doses of DAMGO.

this hyperthermic profile developed in a robust, quadratic fashion, $F(1, 6) = 32.8, p < 0.001$, over testing.

Collectively, the vehicle-pretreated rats exhibited a linearly increasing DAMGO-induced hyperthermia, $F(1, 12) = 102.1$, $p < 0.001$, that ranged from a mean of −0.3°C to a mean of 1.2°C across the saline and high dose of DAMGO. This hyperthermia, however, was potentiated for CA rats, $F(1, 12) = 4.8$, $p < 0.05$. The mean hyperthermia obtained for the 1.0-μg dose of DAMGO in NCA rats was 1.2°C, but exceeded 1.7°C for CA rats. Trend analyses for naltrexone-pretreated rats revealed a negligible dose-dependent DAMGO hyperthermia, $F(2, 24) = 2.69$, $p > 0.05$, and no evidence that cold acclimation influenced naltrexone's effectiveness as an antagonist, $F(1, 12) = 0.41, p > 0.05$.

An overall ANOVA of the maximum elevations of core temperature from baseline, illustrated in Fig. 2, provided a clearer perspective on the robust influence of cold acclimation on DAMGO hyperthermia. In this regard, the peak DAMGO-induced hyperthermia in the vehicle-pretreated rats (mean = 1.4°C) exceeded the hyperthermia in naltrexone-pretreated rats (mean = 0.5°C), $F(1, 24) = 51.1$, $p < 0.001$. Moreover, the peak hyperthermia was dose dependent, $F(2, 48) = 99.5, p < 0.001$, and ranged from a mean = 0.1°C to a mean = 1.7°C. Finally, the Acclimation Condition × Pretreatment × DAMGO Dose interaction, $F(2, 48) = 3.57$, $p < 0.05$, demonstrated that the DAMGO-induced, dose-dependent peak hyperthermia for vehicle-pretreated, CA rats exceeded those obtained by all other groups.

Activity. No main effects or interactions were obtained to link acclimation condition, drug pretreatment, or DAMGO administration (Fs < 1) to the activity profiles observed following DAMGO administration (mean = 44, SE = 4 squares traversed/10 min). However, trend analysis of the Time effect, $F(7, 168) = 31.57$, $p < 0.001$, showed that activity decreased linearly, $F(1, 24) = 104.5, p < 0.001$, from a high of mean = 82 squares traversed/10 min at test onset to mean = 10 squares traversed during the last 10 min of the 80-min test session.

EXPERIMENT 2

Without elevations in thermal set point, DAMGO's hyperthermic action, especially in cold-acclimated rats, could promote heat sensitivity. Increased heat sensitivity evidently contributes to the reduced cold escape observed after 2–4 weeks of continuous (7,24) or intermittent (34) cold acclimation. This notion stems from findings (28) that, if given the opportunity to do so, cold-acclimated rats selected an ambient temperature 8°C cooler than chosen before acclimation without changing body temperature. The few studies to directly assess the effect of cold acclimation on heat sensitivity also reported that cold acclimation lowers the heat tolerance of small mammals (26) and heat accumulation in warm stressed (10,12) or exercising (20) rats. Moreover, cold acclimation produces a vasoconstrictor-dependent hypertension (38). Other vasoconstrictor-dependent forms of hypertension, without the metabolic adapations of cold acclimation, exhibit less heat tolerance (13) and longer durations of heat escape (52) than normotensive controls. These considerations, coupled with the results of Experiment 1 and the interchangeability of behavioral and autonomic means of thermoregulation (37), predict the following. First, central DAMGO or cold acclimation should increase behavioral heat escape relative to that obtained in naltrexone-pretreated or non-cold-acclimated controls. Second, if the heat sensitivity produced by these treatments is cumulative, then DAMGO-induced increases in behavioral heat escape should be markedly potentiated with cold acclimation. Experiment 2 was designed to examine these predictions.

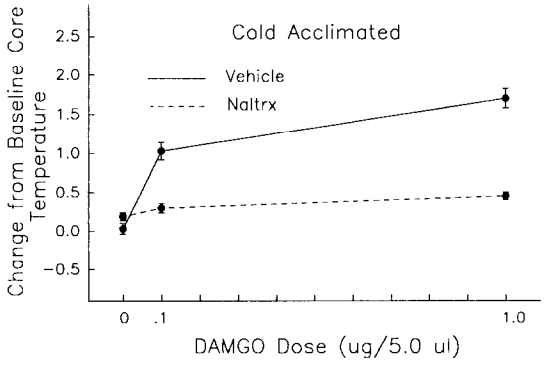

FIG. 2. Mean ± SEM dose-dependent change from baseline core temperature after ICV administration of DAMGO in naltrexone- and vehicle-pretreated cold-acclimated rats.

METHOD

Subjects

Twenty-eight male, Sprague–Dawley rats were used. The animals were housed and fed in a manner identical to that described for Experiment 1.

Apparatus

Convective thermal controller. Operant thermoregulation was assessed in a convective thermal controller (CTC) (52). The CTC is a forced convection system in which the animal is bathed in low-humidity air of controlled temperature and velocity. Air circulates over the animal in an operant chamber, with the temperature being controlled behaviorally. As illustrated in Fig. 3, two continuously circulating air systems are arranged so that one system is circulated through the chamber and the other system is vented through a bypass. When an animal presses a lever, two valves that control the direction of air flow rotate 90°. The air flow circulating through the operant chamber is then diverted through the bypass, whereas the air moving through the bypass is rerouted to the operant chamber. This closed system permits changes in chamber temperature to occur from one stable state to another in only the time it takes the valves to rotate (0.3 s) and air to reach the animal. The components of the CTC are interconnected by an insulated sheet metal duct (i.d. = 15.2 cm). The air temperature is attained by one of two 30.5-cm^2 thermal sources: the hot box containing a 240 VAC heating coil and the cold box housing several rows of copper tubing heat exchangers. Manually operated thermostats control the temperature (±0.5°C) of the heater coils in the hot box or of a duct heater located upstream from the refrigerator freon heat exchanger.

The operant chamber is 48 × 31 × 28 cm and is constructed of insulated sheet metal and Plexiglas. Baffles are located at each end to prevent lamination of air flow. The animal is supported on a 1.6-cm-thick plastic mesh floor that prevents rapid heat exchange, which may occur at the surfaces of animals exposed to more conductive materials. The operant manipulandum measuring 5 × 3 × 1 cm is thermally insulated, and is located 2 cm above the chamber floor on the wall opposite the chamber door. A feces trap, containing sawdust, is placed beneath the floor, and is replaced for each animal. The ambient temperature of the CTC was 37°C, and the thermal reinforcement was a 17°C air flow through the chamber.

Procedure

Pretest. The rats arrived on day 1. As in Experiment 1, half (n = 14) were assigned to the CA condition and began 26 consecutive days of exposure to 5°C in the walk-in cooler. The remaining animals (n = 14) were assigned to the NCA control condition. On day 3 all animals began a heat escape (37°C) shaping procedure. Shaping the operant thermoregulatory response was accomplished through successive approximations. This procedure involved placing the rat in the CTC during a heat escape challenge (37°C) and using cool air (17°C) as reinforcement for those responses that approximated the appropriate lever press, until the animal could respond unassisted for the cool air without becoming hyperthermic. Once this level of proficiency was obtained, the animal was left in the heat challenge situation for an additional 6 h during which time a stable response rate was attained. A stable response rate was defined as a) at least 350 s of responding for each 30-min interval, b) consistent response patterns, and c)

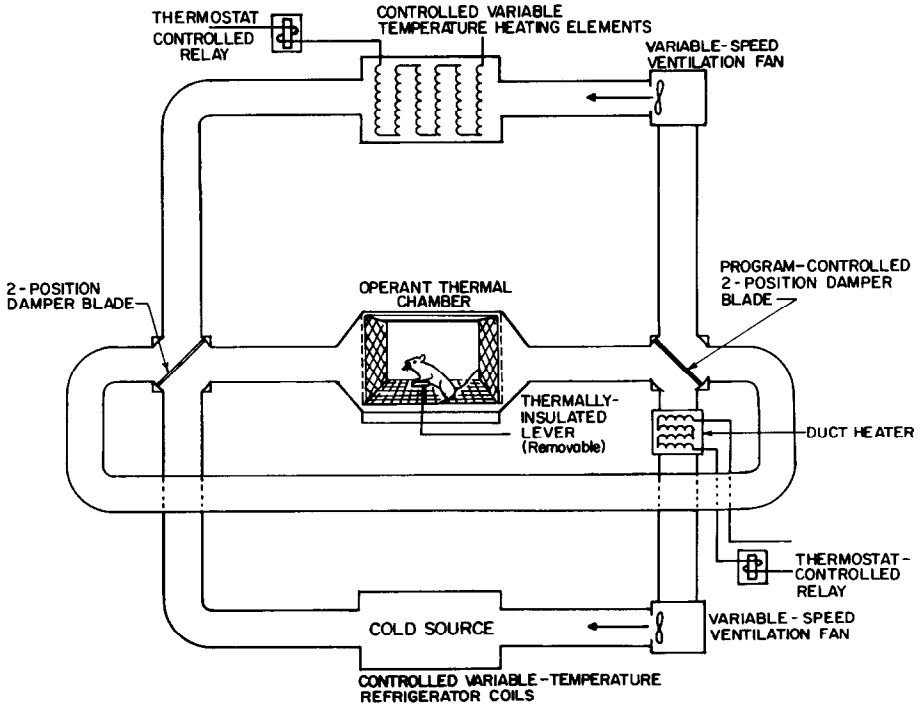

FIG. 3. Schematic diagram of the Convective Thermal Controller used to measure heat escape.

no evidence of hyperthermia at the end of the shaping session. One subject required longer than 48 h to attain criterion and was excluded from the study.

Surgery. Surgical procedures included ICV cannulation and sialoadenectomy. These surgeries were performed under the same conditions and in the same manner as described for Experiment 1.

Behavioral heat (37°C) escape testing. The CA and NCA rats that were successfully shaped were then assigned to either a VEH-DAMGO or a NLX-DAMGO test condition. The drug administration procedure was similar to that used in Experiment 1: the VEH-DAMGO group was given an IP injection of 0.15 M saline followed 40 min later by an ICV injection of either saline or one of two doses of DAMGO. The NLX-DAMGO group was given a naltrexone injection (2 mg kg^{-1}, IP) followed 40 min later by an ICV injection of either saline or one of the two doses of DAMGO. Accordingly, the experiment consisted of four groups each with seven subjects: the CA-VEH-DAMGO, NCA-VEH-DAMGO, CA-NLX-DAMGO, and the NCA-NLX-DAMGO group.

Twelve hours before testing, rats in both the NCA and CA groups were relocated into a separate room as described for Experiment 1. On days 21–25 three sessions of behavioral heat (37°C) escape testing began, with rats tested on alternating days. Two rats were tested per day commencing at 0830 h, and each testing session was conducted as follows. Following the IP injection of either 0.15 M saline or naltrexone HCl (2 mg kg^{-1}), in a volume of approximately 0.25 ml 100 g^{-1}, the rat was placed in the CTC with the ambient temperature set at 37°C. The animals were then allowed 40 min to equilibrate thermoregulatory responding. The last 30 min of this period constituted the preinjection baseline. Following the preinjection baseline, 5 μl of either saline or one of the two doses of DAMGO was administered ICV. As described in Experiment 1, the ICV injections were given over a 1-min period using a 10-μl syringe (Hamilton, Reno, NV) placed through the guide cannula. Readings of core temperature were taken before and immediately after each test session. Duration and frequency of lever press were accumulated over 10-min periods during the 80-min test session. Following each testing session, the animal was removed from the CTC and replaced in its home cage. This procedure was repeated on days 23 and 25, such that by the end of the day 25 testing session, each rat had received IP vehicle or naltrexone followed 40 min later by ICV saline and both doses of DAMGO. The order of drug adminstration was counterbalanced throughout the experiment and the naltrexone and DAMGO solutions were prepared as described in Experiment 1. Body weights were recorded before each experimental session. Core temperatures were recorded before and after each experimental session using a YSI thermal probe (Model 409) lubricated with mineral oil and inserted rectally 6 cm. Cannula placements were verified by injecting India ink ICV and then examining the brain postmortem.

Statistical Analyses

Baseline values for body weight and core temperature were assessed using a 2 × 2 (Acclimation Condition × Drug Pretreatment) factorial ANOVA, whereas baseline values for lever press duration and frequency, obtained across four 10-min intervals, were analyzed via 2 × 2 × 3 × 4 (Acclimation Condition × Drug Pretreatment × Test Day × Sampling Time) mixed factorial ANOVAs with repeated measures on Test Day and Sampling Time. Post-DAMGO values for core temperatures were analyzed by a 2 × 2 × 3 × 2 (Acclimation Condition × Drug Pretreatment × DAMGO Dose × Pre : Posttest) mixed factorial ANOVA with repeated measures on DAMGO Dose and Pre : Posttest, whereas values for lever press duration and frequency, obtained across eight 10-min intervals, were analyzed by 2 × 2 × 3 × 8 (Acclimation Condition × Drug Pretreatment × DAMGO Dose × Sampling Time) mixed factorial ANOVAs with repeated measures on DAMGO Dose and Sampling Time. Trend analyses for main effects and interactions followed the ANOVAs, with alpha levels for all analyses set at $p < 0.05$.

RESULTS

Baseline Values

Body weight. Pretest body weights of cold-acclimated rats (mean = 218.6, SE = 5.4 g) tended to be lower than the weights of non-cold-acclimated rats (mean = 226.3, SE = 3.5 g), although this difference was not significant, $F(1, 24) = 1.31$, $p > 0.05$. The body weights of those rats eventually assigned to different drug pretreatment conditions contributed little to group main effects or interactions (Fs < 1).

Core temperature. Baseline core temperatures (mean = 37.2, SE = 0.3°C) were independent of both the acclimation conditions and the eventual assignment to drug pretreatment condition (Fs < 1).

Lever press duration and frequency. The influence of cold acclimation on duration (mean = 205.5, SE = 5.7 s/10 min) or frequency (mean = 10.1, SE = 3.7 responses/10 min) of baseline heat escape responding was not significant. Both variables, however, changed in a reciprocal manner with time. Analysis of Sampling Time over the 40-min session (Fig. 4) revealed that baseline heat escape durations progressively increased, $F(3, 72) = 27.14$, $p < 0.001$, as response frequencies decreased, $F(3, 72) = 32.55$, $p < 0.001$. The significant quadratic components obtained for both these main effects indicated, however, that these profiles reached asymptote shortly before DAMGO testing. The response profiles of those rats eventually assigned to different drug pretreatment conditions contributed little to group main effects or interactions (Fs < 1).

Post-DAMGO Values

Core temperature. The core temperatures obtained after ICV injections and after the heat escape testing sessions (mean = 37.4, SE = 0.3°C) were slightly elevated above the baseline core temperature, $F(1, 24) = 8.42$, $p < 0.01$, but no other main effects or interactions were found with either drug pretreatment or DAMGO dose (Fs < 1). Cold-acclimated rats also tended to exhibit slightly lower core temperatures (mean = 37.3, SE = 0.3°C) than non-cold-acclimated controls (mean = 37.4, SE = 0.3°C), but this effect only approached statistical significance, $F(1, 24) = 3.75$, $p < 0.06$.

Lever press duration and frequency. Figure 4 suggests that the influence of cold acclimation on heat escape was pronounced. Specifically, the response durations for cold-acclimated rats (mean = 271.3, SE = 9.0 s/10 min) were considerably longer than those exhibited by the non-cold-acclimated controls (mean = 224.0, SE = 7.9 s/10 min), $F(1, 24) = 16.45$, $p < 0.001$. There was a nonsignificant tendency, $F(2, 48) = 2.65$, $p < 0.08$, for this CA-induced potentiation of heat escape duration to be even greater in saline-pretreated rats at the highest dose of DAMGO. No other

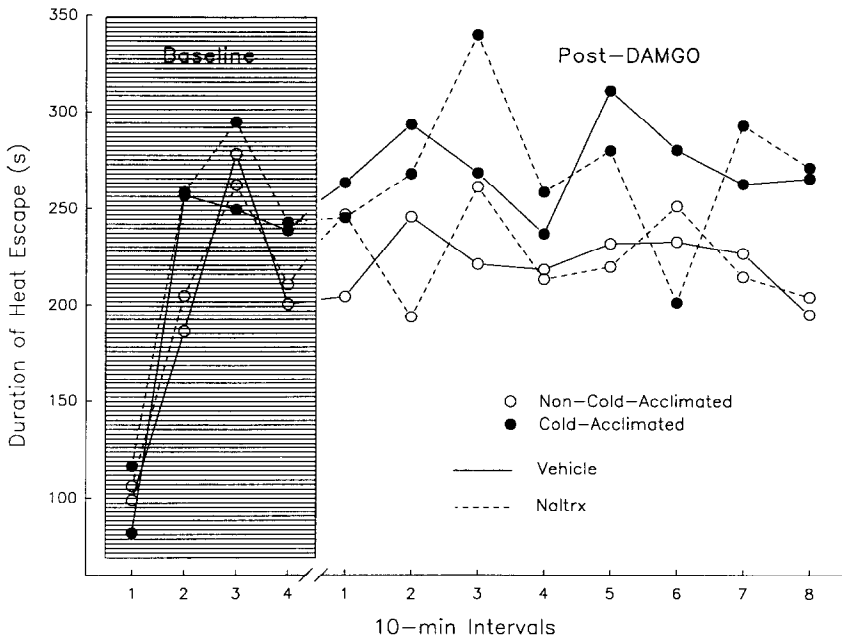

FIG. 4. Mean duration of heat escape for 40 min preceeding and 80 min following ICV administration of DAMGO in naltrexone- and vehicle-pretreated rats.

reliable main effects or interactions on lever press duration were obtained. The frequency of lever pressing for heat escape was independent of acclimation condition, drug pretreatment, and dose of DAMGO administered (Fs < 1).

DISCUSSION

Throughout both experiments baseline core temperatures were not influenced by either cold acclimation or naltrexone pretreatment, and no group differences in body weight existed during testing. In non-cold-acclimated rats, centrally administered DAMGO promoted hyperthermia, the amplitude and latency of onset of which was dose dependent and similar to previous reports with unrestrained, non-cold-acclimated rats (44). The naltrexone reversibility of this hyperthermia also supports the naloxone-reversible hyperthermia observed with central injections of DAMGO (44) or related μ-opioid agonists (46,48). The principal finding in Experiment 1, however, was that DAMGO's hyperthermic action was markedly potentiated with cold acclimation, exhibiting a faster onset, greater peak amplitude, and longer duration than without cold acclimation. This potentiated hyperthermia was naltrexone reversible, but slightly less so than in non-cold-acclimated rats, suggesting that, like naloxone (44), naltrexone's ability to block DAMGO hyperthermia is dose dependent. There was no indication that, with or without cold acclimation, DAMGO hyperthermia was influenced by any detectable patterns or trends in locomotion. Accordingly, the naltrexone reversibility of cold acclimation-induced potentiation of DAMGO hyperthermia, coupled with reports that cold acclimation potentiates β-endorphin hyperthermia (47,53), not only indicates a thermogenic role of central μ-opioid receptors but suggests that this role is strongly influenced by central adaptations that emerge during cold acclimation in unrestrained rats.

DAMGO's hyperthermic action, the potentiation of this action with cold acclimation, and indications that cold acclimation exacerbates heat sensitivity suggested that cold acclimation and central DAMGO would enhance heat escape responding, functioning alone or in combination. Confirming these expectations in Experiment 2 was the finding that vehicle- and naltrexone-pretreated cold-acclimated rats exhibited a stable increase in the duration of heat escape. This profile emerged shortly before the DAMGO injections and persisted throughout the testing session. As found elsewhere (17,28), body temperatures were normal in cold-acclimated rats if recorded in a neutral ambient temperature. The increased heat escape, alone or viewed with reports of reductions in cold escape (7,24,34), can be easily reconciled with findings that cold-acclimated rats prefer cooler temperatures when placed on thermal gradients (28). From these observations it seems that cold acclimation not only improves cold tolerance, thereby reducing reliance on behavior to prevent hypothermia, but also jeopardizes heat tolerance, thereby increasing reliance on behavior to offset heat loads. However, in contrast to these expectations, DAMGO exerted no influence on heat escape responding, even in cold-acclimated rats. Accordingly, what results is an apparent double dissociation: cold acclimation produces a naltrexone-resistant increase in heat escape responding while exerting no effect on body temperature except through a marked potentiation of DAMGO hyperthermia. In contrast, DAMGO produces a naltrexone-sensitive, μ-opioid receptor-mediated elevation in body temperature, whereas neither this hyperthermia nor the potentiation of this hyperthermia with cold acclimation influences heat escape.

Why DAMGO hyperthermia, especially in cold-acclimated rats, failed to increase heat escape is unclear. Heat escape may not be sensitive enough to detect DAMGO-induced increases in heat sensitivity. However, this notion is difficult to recon-

cile with the sensitivity to cold acclimation obtained in this study and the sensitivity to other temperature-related manipulations obtained in other studies (27,52). Alternatively, DAMGO-induced increases in thermal set point or analgesia could have buffered increases in heat escape. In this regard, one report measured ambulation on a thermal gradient and found that central DAMGO injections increased both body temperature and preferred ambient temperature (45). However, this apparent elevation in thermal set point was detected only when the data had been adjusted for differences in latency to reach peak hyperthermia and, even then, was quite transient and was followed by a preference for a cool environment. Through elimination emerges perhaps the most plausible explanation: classic μ-opioid-mediated analgesia may have buffered increases in heat sensitivity, and thereby heat escape, that would have otherwise accompanied both DAMGO hyperthermia and the potentiation of this hyperthermia with cold acclimation. This explanation remains to be verified.

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