ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 2, pp. 219–225, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00071-8

Effect of Restraint on Drug-Induced Changes in Skin and Core Temperature in Biotelemetered Rats

BRUCE E. WRIGHT AND MICHAEL J. KATOVICH[1]

Department of Pharmacodynamics, School of Pharmacy, University of Florida, Health Science Center, P.O. Box 100487, Gainesville, FL 32610

Received 7 July 1995; Revised 3 January 1996; Accepted 9 January 1996

WRIGHT, B. E. AND M. J. KATOVICH. *Effect of restraint on drug-induced changes in skin and core temperature in biotelemetered rats.* PHARMACOL BIOCHEM BEHAV **55**(2) 219–225, 1996.—Temperature homeostasis is modulated by a number of neuroendocrine control systems. Both angiotensin II and isoproterenol have been shown to increase skin temperature. Withdrawal from opioid dependence using naloxone also results in an increased skin temperature and a decreased body core temperature. The effects of restraint stress on these tail skin temperature responses is unknown. We tested the effect of restraint or free movement on tail skin and core temperature responses to three thermoregulatory substances: isoproterenol and angiotensin II in naive rats and naloxone in morphine-dependent rats. In each case restrained rats had significantly lower baseline tail skin temperatures than free moving rats. Baseline core temperatures were not different between restrained and free moving animals. Each agent produced significant acute increases in tail skin temperatures. Restraint did not affect these responses. Both angiotensin II and naloxone also produced significant decreases in core temperatures that were not altered by restraint. This study is the first to show that radiotelemetry can be used to measure tail skin temperatures in rats. The results of this study show that when using three different thermoregulatory agents restraint failed to affect either baseline temperatures or maximal responsiveness to the agents in a detrimental manner. The lack of impairment of temperature changes due to restraint in these studies also validate previous studies that had used restraint in measuring core and tail skin temperatures in rodents. **Copyright © 1996 Elsevier Science Inc.**

Temperature regulation Core temperature Tail skin temperature Isoproterenol Angiotensin II Naloxone Biotelemetry Minimitters

FOR homeothermic animals, the purpose of temperature regulation is to maintain a constant core temperature such that the function of vital organs (heart, liver, etc.) is not compromised. Under many circumstances this is accomplished over long-term changes in basal metabolic rate and spontaneous activity, both of which affect heat production. Heat loss is controlled by several mechanisms, one of which is the control of skin temperature. This is especially true in the rat, where acute increases or decreases in tail skin temperature can either result from or result in corresponding or contrasting changes in core temperature, depending on the situation (22). Over the long term, changes in skin temperature serve to interact with metabolic rate changes to maintain the core temperature at some homoeostatically determined point.

Temperature is modulated by a number of neuroendocrine substances. These include catecholamines directly and indirectly associated with sympathetic nervous stimulation (9,11,14), peptide hormones such as angiotensin II (25), and opioids (14–19,23). Steroid hormones such as corticosterone (15) and estradiol (14) also have been implicated in alteration of skin temperature. In general catecholamines, angiotensin II, and glucocorticoids, increase skin temperature (9,11,14,16,25), while estrogens prevent these increases (14). Depending on dose, opioids can produce either an increase or decrease in core temperature (5). Acute opioid withdrawal with naloxone produces an acute increase in skin temperature and decrease in core temperature (17,23).

The ability of both catecholamines and glucocorticoids to affect tail skin temperature begs the question, what role does stress play in the control of tail skin temperature? In almost all studies on core temperatures performed on rats, restraint devices of one type or another have been used (1,10,20). It

[1]To whom requests for reprints should be addressed.

has been suggested that this stress increases core temperatures (1,21) in spontaneously hypertensive rats. The effects of restraint on temperature in normal rats, and the modulatory effect restraint stress has on other agents' ability to change core temperature, is not as certain. In one study (20), restraint stress improved the ability of morphine to decrease core temperature. In another, it decreased the ability of CRF to do the same thing (10). In addition, in many studies we and others used rectal probes to monitor core temperatures (14,20,25). These temperature probes themselves have been shown to acutely increase core temperatures, particularly when inserted intermittently in spontaneously hypertensive rats (1), compared to those observed using implanted abdominal radiotelemetry probes. It should be noted, however, that over a longer period of time these rats may be able to adjust to rectal probes (21). In addition, another study showed that core temperature data from abdominal radiotelemetric probes and rectal probes produce compatible results in normal rats (6).

Whether tail skin temperature is as susceptible to restraint stress-induced changes as core temperature is unknown. All studies in which tail skin temperatures were recorded have required restraint devices to prevent the rats from removing the probes. Radiotelemetry probes would eliminate this requirement and make it possible to study the effect of restraint on tail skin temperature. However, to our knowledge there are no published reports in which radiotelemetric probes have been used to measure tail skin temperature.

The aim of this study was to evaluate the role of restraint in temperature regulation. We hypothesized that restraint would increase the basal skin temperature and, thus, make the maximal change in skin temperature in response to various pharmacological agents appear smaller in magnitude. To test this hypothesis, we developed a novel protocol in which radiotelemetric probes were used to measure tail skin temperatures. To our knowledge, we are the first to use telemetry to measure skin temperature.

METHOD

General Methods

Male Sprague–Dawley rats (Charles Rivers, Wilmington, DE) were individually housed in stainless steel hanging cages and provided rodent chow (#5001, Ralston-Purina, St. Louis, MO) and water ad lib. They were maintained on a 14 L:10 D cycle (lights on from 0700 to 2100 h) in a certified animal care facility. "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. The protocol was approved by the university IACUC.

Surgery

Each rat was implanted with a biotelemetric radio unit fitted with a silastic temperature probe (Model VM-FH Mini-Mitter, Mini-Mitter Co., Sunriver, OR) to measure temperature. The thermistor tip was located 2–7 cm from the transducer/transmitter module. The entire assembly was sealed by either a plastic sheath alone or a sheath plus an additional wax coating over the transmitter module.

Minimitters were sterilized prior to implantation by immersion in glutaraldehyde (10% w/w, Sigma). They were rinsed three times in sterile distilled water immediately prior to implantation. The rats were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) during implantation. When a minimitter was implanted into the abdomen (in order to measure core temperatures), it was left free in the abdominal cavity. The abdominal wall was stitched closed and the skin above was closed with wound clips. When a minimitter was implanted into the tail, an incision was made in the center of the back. A cavity was created in the lower back from the incision site, and below the cavity a channel was threaded under the tail skin with a round tip probe (Mini-Mitter Co.). Using sterile forceps, the thermistor tip was teased into that channel. The transmitter assembly was left in the cavity in the lower back. The transmitter and tip were held in a subdermal pocket by placing sterile wound clips just dorsal to the transmitter assembly such that the skin at the incision site itself was not under tension and the probe tip could not slip out from under the tail skin. The incision was closed with sterile wound clips and allowed to heal for at least 6 days before the first temperature measurements were recorded.

In animals made tolerant to morphine (Experiments 2 and 3), each rat was anesthetized with Metofane. A pellet containing 75 mg morphine sulfate (Mallincrodt, St. Louis, MO) was implanted subcutaneously in the upper back. The skin was closed with wound clips. Two days later each rat received two additional morphine pellets. Two days after that the rats were considered to be tolerant to morphine (3,24).

For each experiment temperature data were analyzed by both two-way and one-way analyses of variance, the latter using Scheffe's F-test (or, when appropriate, Fisher's PLSD) as a post hoc test. A p-value of less than 0.05 indicated significant differences between groups.

Experiment 1: Effect of Restraint on Isoproterenol and Angiotensin II-Stimulated Temperature Changes

Twelve Sprague–Dawley rats (261–292 g) had minimitter probes implanted in their tails. After 7 days each rat was weighed and randomly placed into either an ordinary wire restraining cage with an acrylic floor or a free-moving environment. This free moving environment consisted of an open-topped cardboard box (about 25 by 30 cm, 17 cm high) into which several dozen holes had been cut. Each box or cage was then placed on a receiver calibrated to its own radio transmitter. The rats' tail skin temperatures could be recorded, transduced into radio signals, and received by the external devices. The frequency of each signal could then be converted into temperature using the Dataquest III program (Mini-Mitter Co., Sunriver, OR) on an IBM PC. All temperature data was processed for a weighted mean temperature for 5-min increments. All measurements in this experiment took place between 1400 and 1600 h.

Following a control period of approximately 90 min, each rat was injected subcutaneously with vehicle (saline, 1 ml/kg). The time of each injection was recorded and the temperatures for the last 25 min prior to injection (the "late" control period) were used to determine basal skin temperatures. Tail skin temperatures were monitored for 60 min after this injection. Rats were then removed, weighed, and returned to single housing. On the following day the same procedure was followed except that isoproterenol (100 μg/kg, SC) (Isuprel, Winthrop Pharmaceuticals, NY) was administered instead of saline. On a subsequent day angiotensin II (200 μg/kg, SC) (Research Biochemicals International, Natick, MA) was administered. Transmitters were subsequently removed, resterilized, and placed in the abdominal cavity of each rat. Four days later the rats were again randomly placed in free and restrained groups and treated as above except that the angiotensin II injection followed the isoproterenol treatment by 3

days. Core temperatures were monitored for 60 min following injection of vehicle or agonist.

Experiment 2: Effect of Restraint on Naloxone-Induced Tail Skin Temperature Changes in Morphine-Dependent Sprague–Dawley Rats

Twelve Sprague–Dawley rats, initially weighing 175–199 g, were used. All rats were fitted with minimitters and made morphine dependent. Testing took place between 0800–1100 h. On the first day of testing each rat was weighed and randomly placed into restraining cages or cardboard boxes as described in Experiment 1. Within each restraint environment, rats were sorted by matching body weights into saline (1 ml/kg) or naloxone (1 mg/kg, Sigma) treatment groups. Thus, there were a total of four groups (free-moving + saline, restrained + saline, free-moving + naloxone, and restrained + naloxone, $n = 3$ per group). Following the control period rats were injected with either vehicle or naloxone, and tail skin temperatures were recorded for 60 min. On the following day, each rat was placed in the opposite restraint environment than it had experienced the day before. Each rat was given the opposite treatment as well, such that all animals experienced both saline and naloxone treatments, and free-moving and restrained environments exactly once during the study. Repeated measures was used to confirm a lack of conditioning effects between day 1 and day 2. In this study data from both days are therefore combined in the results.

Experiment 3: Effect of Restraint on the Core (Abdominal) Temperature Response to Naloxone in Morphine-Dependent Rats

Twelve rats weighing 322 ± 6 g were used. Minimitters were implanted into their abdomens. Five days later, basal core temperatures were measured in free-moving conditions, after which the rats were made morphine dependent. Once this had occurred, each rat was randomly assigned to free-moving and restrained groups ($n = 6$ per group). Following a control period all rats were then injected with naloxone (1 mg/kg, SC). Core temperatures were measured before and after treatment with naloxone. Measurements were taken between 1400–1600 h. This protocol was followed the next day except saline was administered to each rat.

RESULTS

Experiment 1: Effect of Restraint on Tail Skin and Core Temperature Responses to Isoproterenol and Angiotensin II in Naive Sprague–Dawley Rats

The effects of restraint on tail skin and core temperatures are shown in Figures 1–3. Figure 1 displays the effect of restraint and isoproterenol on mean change in tail skin temperatures over time. A significant restraint effect was displayed during the late control period. Contrary to our hypothesis, however, free moving rats had significantly higher mean basal tail skin temperatures than did restrained rats (30.35 ± 0.41 vs. 28.57 ± 0.36°C for free and restrained rats, respectively; $p < 0.01$). Isoproterenol administration resulted in an initial but transient fall in tail skin temperature followed by a significant elevation in skin temperature. By 25 min after the isoproterenol injections, tail skin temperatures displayed a significant drug effect that remained through 60 min after treatment. However, because two-way analysis of variance revealed no interaction between the drug and restraint effects, there was

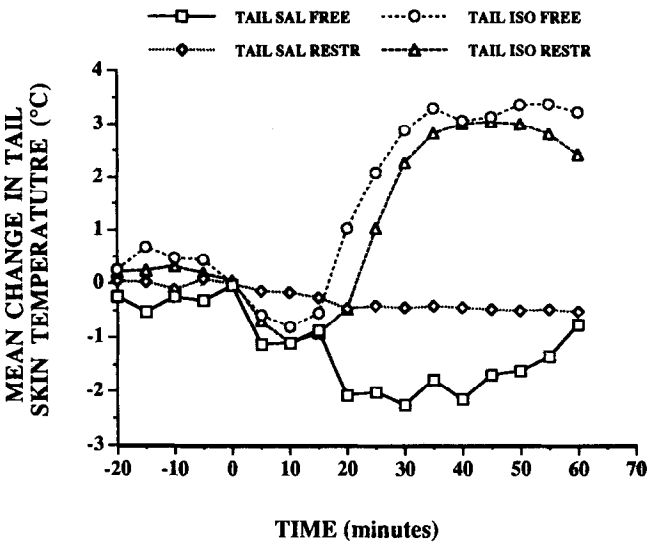

FIG. 1. Effect of restraint and isoproterenol on the mean change in tail skin temperatures of naive animals over time. At time 0, either vehicle (saline, 1 ml/kg SC) or isoproterenol (100 μg/kg) was administered to Sprague–Dawley (SD) rats. Data represent mean changes in temperature from baseline for each group at each time point. n = 4–6 per group at each time. Significant ($p < 0.05$) isoproterenol effects were observed 25–60 min following administration of the agent.

no selective effect on any one restraint plus drug group (e.g., restrained rats given isoproterenol) compared to any other group. This suggests that restraint in and of itself did not modify the effect of isoproterenol on tail skin temperature changes even though the baseline temperatures between restrained and free-moving rats were different. At a representative time, 45 min posttreatment, the change from baseline in tail skin temperatures in isoproterenol-treated rats were 3.10 ± 0.58 and 3.00 ± 0.42°C for free-moving and restrained animals, respectively.

With respect to core temperatures, there was no significant difference during the pretreatment period between free-moving and restrained rats (36.47 ± 0.41 vs. 35.98 ± 0.49°C). There was also no significant effect of either isoproterenol or restraint on core temperatures in these animals at any time during the 60-min treatment period.

Figure 2 displays the effect of restraint and angiotensin II on the mean change in tail skin temperatures over time. During the late control period, free-moving rats had significantly higher tail skin temperatures than did restrained rats (30.69 ± 0.29 vs. 29.32 ± 0.46°C for free and restrained groups, respectively). However, there was also a "treatment" effect on basal skin temperature by two-factor ANOVA that was apparently due to a difference between AII-restrained and saline-restrained animals. Despite this complicating factor, administration of angiotensin II produced a rapid and significant increase in tail skin temperature that was maximal at 10 min postinjection. At this time the change in tail skin temperatures were similar for the two AII-treated groups from their baselines (2.2 ± 0.42 and 2.15 ± 0.23°C for the AII-free and AII-restrained groups, respectively) and were significantly greater than that observed with the two saline treated groups (−1.10 ± 0.68, and −0.21 ± 0.21°C for saline-free, and saline-restrained groups, respectively). As with isoproterenol treatment, restraint did not affect the surge in tail skin temperature induced by angiotensin II.

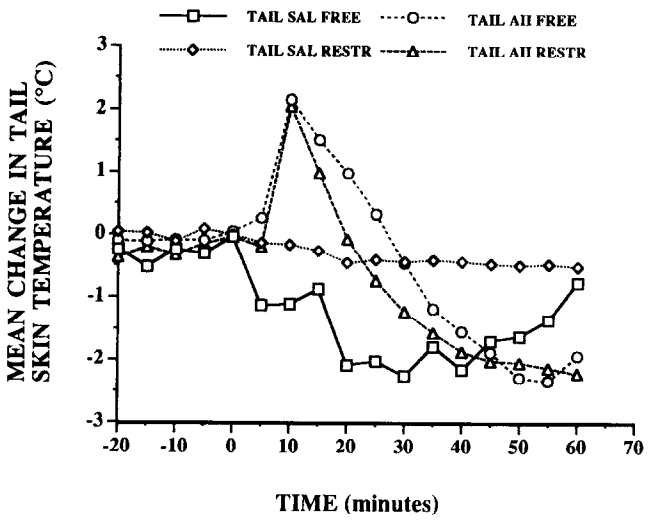

FIG. 2. Effect of restraint and angiotensin II on the mean change in tail skin temperature of naive animals over time. At time 0, either vehicle (saline, 1 ml/kg SC) or angiotensin II (200 μg/kg) was administered to Sprague–Dawley (SD) rats. Data represent mean changes in temperature from baseline for each group at each time point. n = 4–6 per group at each time.

Figure 3 displays the effect of restraint and angiotensin II on the mean change in core temperatures over time. During the late control period there was no significant effect of restraint on core temperatures (36.52 ± 0.42 vs. 36.00 ± 0.39°C for free-moving and restrained rats, respectively). Following angiotensin II administration there was a small but significant decrease in core temperature from 20 to 55 min after the agent was injected. At 50 min postinjection the changes in core temperatures were 0.62 ± 0.30, 0.22 ± 0.36, −0.57 ± 0.25,

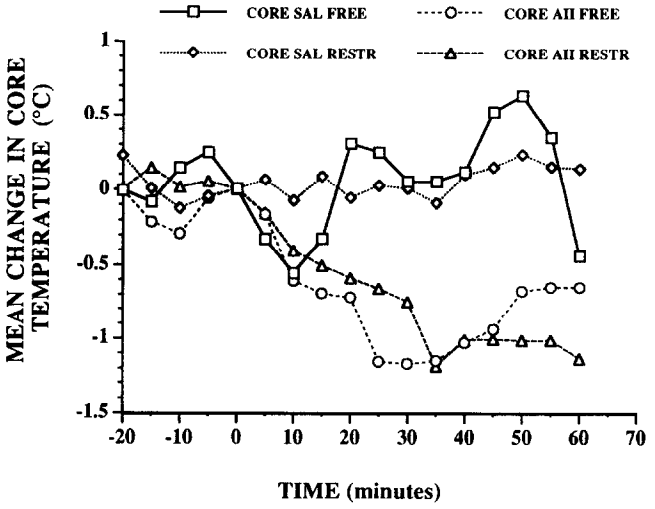

FIG. 3. Effect of restraint and angiotensin II on the mean change in core temperature of naive animals over time. At time 0, either vehicle (saline, 1 ml/kg SC) or angiotensin II (200 μg/kg) was administered to Sprague–Dawley (SD) rats. Data represent mean changes in temperature from baseline for each group at each time point. n = 4–6 per group at each time. Significant ($p < 0.05$) angiotensin II effects were observed 20 to 55 min following administration of the agent.

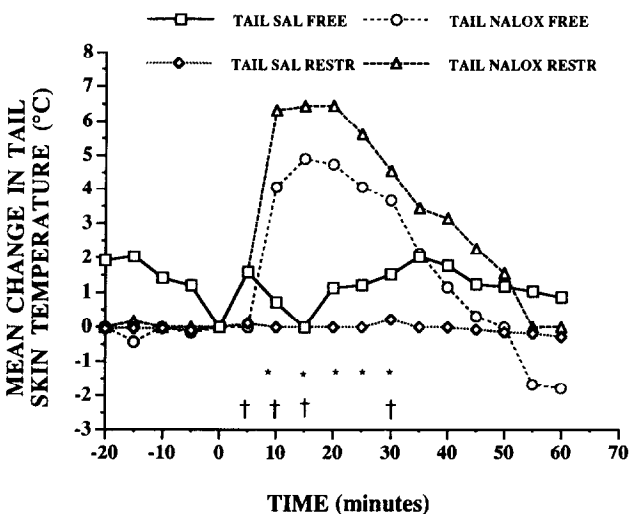

FIG. 4. Effect of restraint and naloxone on the mean change in tail skin temperature of morphine-dependent animals over time. At time 0, either vehicle (saline, 1 ml/kg SC) or naloxone (1 mg/kg) was administered to morphine-dependent Sprague–Dawley (SD) rats. Data represent mean changes in temperature from baseline for each group at each time point. n = 4–6 per group at each time. Two-way analysis of variance was used. For each time, significant ($p < 0.05$) naloxone (*) and/or restraint (+) effects are noted.

and −1.05 ± 0.35°C for saline-free, saline-restrained, AII-free, and AII-restrained rats, respectively. There was no significant difference in temperature changes between the free-moving +AII and restrained +AII groups. However, there were significant differences between AII restrained and saline restrained groups (p = 0.01) and between AII free and saline free groups (p = 0.001). Together, these results indicate that angiotensin II induced significant decreases in core temperatures whether animals were restrained or not.

Experiment 2: Effect of Restraint on Tail Skin Temperatures in Morphine-Dependent Rats

The effect of restraint and naloxone on the mean change in tail skin temperature over time are displayed in Fig. 4. The median basal (late control period) tail skin temperatures of the free moving group and the restrained group were 30.11 ± 0.48 and 27.58 ± 0.56°C, respectively. This was a significant difference (p = 0.0024). As expected, the vehicle (saline) did not affect tail skin temperatures significantly. In contrast, naloxone treatment produced a pronounced and similar increase in tail skin temperature in both restrained and free-moving rats. The effect was strongest at 15 min after injection. The mean changes in tail skin temperatures at this time were −1.38 ± 0.78, 0.34 ± 0.75, 5.19 ± 0.73, and 6.26 ± 0.42°C for the saline-free, saline-restrained, naloxone-free, and naloxone-restrained animals, respectively. Two-way analysis of variance determined both a significant treatment and restraint effect, but there was no significant interaction, indicating that no one group (e.g., restrained + naloxone) experienced a selective effect on temperature compared to any other. This was confirmed by one-way analysis of variance (Fig. 5). Although the increase in tail skin temperatures appeared to be slightly higher in restrained vs. free-moving animals, they were not statistically different. The stimulatory effect of naloxone on tail skin temperatures lasted from 10 to 30 min postinjection (Fig. 4).

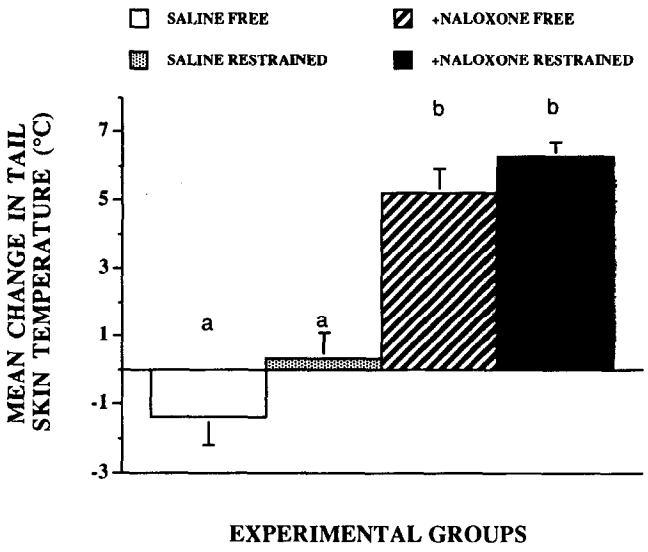

FIG. 5. Effect of restraint and naloxone on the mean change in tail skin temperature 15 min after administration of either vehicle (saline, 1 ml/kg SC) or naloxone (1 mg/kg) to morphine-dependent SD rats. Data represent mean changes in temperature from each group's individual baselines (± SEM). $n = 5$ or 6. Groups sharing letters are not significantly different from one another ($p > 0.05$).

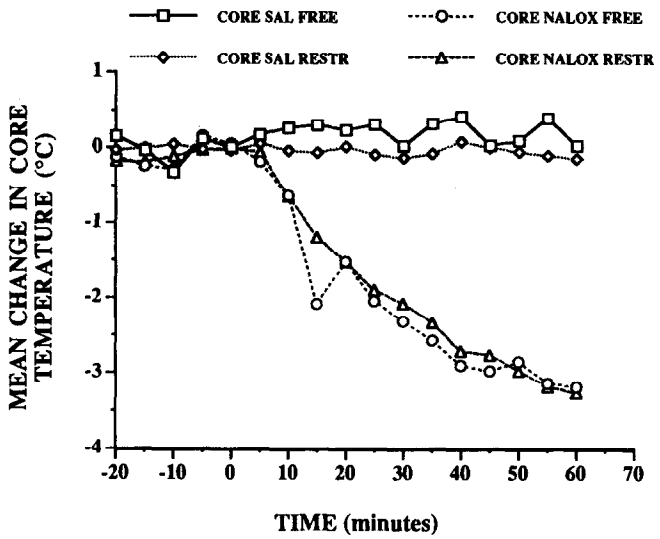

FIG. 6. Effect of restraint and naloxone on the mean change in core temperature of morphine-dependent animals over time. At time 0, either vehicle (saline, 1 ml/kg SC) or naloxone (1 mg/kg) was administered to morphine-dependent Sprague–Dawley (SD) rats. Data represent mean changes in temperature from baseline for each group at each time point. $n = 4$–6 per group at each time. Significant ($p < 0.05$) treatment effects were observed 15 through 60 min after administration of naloxone in the morphine-dependent animals.

Experiment 3: Effect of Restraint on the Core Temperature Response of Morphine-Dependent Rats to Saline or Naloxone

The effect of restraint and naloxone on the mean change in core temperatures over time in morphine-dependent animals is shown in Fig. 6. Baseline (late control period) core temperatures (mean 38.33 ± 0.26°C) did not differ from one another, but they were significantly higher ($p < 0.0001$) than they had been prior to induction of morphine tolerance (36.21 ± 0.25°C). Core temperatures had significantly declined by 15 min after naloxone injection in these animals. These effects were strongest at 60 min after injection (the last time point measured). However, there were no significant effects of restraint either prior to treatment or at any point following treatment. At 60 min after treatment, body core temperatures had changed by 0.12 ± 0.23, −0.12 ± 0.18, −3.24 ± 0.39, and −3.30 ± 0.29°C for saline-free, saline-restrained, naloxone-free, and naloxone-restrained groups, respectively.

DISCUSSION

In this study we had hypothesized that the stress associated with restraint would increase both core temperatures and tail skin temperatures in rats. We had also hypothesized that restraint stress would significantly reduce the magnitude of both skin temperature increases and core temperature decreases to a variety of pharmacological stimuli in naive and morphine-dependent Sprague–Dawley rats. In general, the results, of these studies contradict both hypotheses. Restraint stress did not significantly alter baseline (pretreatment) core temperatures of either naive or morphine-dependent rats. In all three tail skin temperature/drug paradigms, the effect of restraint on baseline temperatures was the opposite of what we had proposed. Restrained animals had significantly lower basal skin temperatures than did free-moving animals. Following drug treatment, significant increases in tail skin temperature were observed with all three agents (isoproterenol, angiotensin II, and naloxone). Restraint did not significantly reduce the maximal surge in tail skin temperature in any of these studies. In two of three studies in which core temperatures declined significantly after drug treatment, restraint did not affect these responses either. The results, therefore, demonstrate that restraint does not exert a deleterious effect on either basal core and tail skin temperatures, or maximal responsiveness to several known thermoregulatory agents in Sprague–Dawley rats.

The radiotelemetry method used to measure tail skin temperature in these studies appears to measure slightly higher baseline temperatures in restrained rats than is typical of those found using more classical techniques (8). This approximately 2 to 3°C difference is to be expected, because unlike other techniques, the thermistor tip lies under, not over, the tail skin. However, this is not necessarily a problem, as long as agents or stimuli that cause significant temperature changes using one method also cause significant changes in the same direction using the other method. Again, this appears to be the case, and furthermore, both naloxone and isoproterenol produced tail skin temperature changes in restrained animals that were quantitatively similar to those seen using more classical techniques (7,12,13,18).

The discovery that under baseline conditions, free-moving rats displayed significantly higher tail skin temperatures than did restrained animals, was surprising. However, there are two possibilities that might explain the variation. First, it had been reported that restraint interferes with thermic responses by preventing posture changes (20). In the current studies, many free-moving rats preferred to curl up in one corner of their box, others laid with their tails under their bodies, and still others did both. Either of these behaviors would be likely to result in a higher temperature microenvironment for free-moving rat tails than for restrained rat tails, which were always

directly exposed to ambient air. Because physically dislodging free-moving rats from heat-trapping postures would by definition be stressful, it proved nearly impossible to prevent these behaviors. Still, because the maximal temperature changes after drug administration did not significantly differ between free-moving and restrained rats, their postural differences proved to be of minor importance.

A second reason why free-moving rats might have had higher basal tail skin temperatures may involve their encountering a novel environment that resulted in higher temperatures during the experiment. After all, the rats had never been in the cardboard boxes before. Although the authors consider it unlikely that the cardboard boxes produced a unique stress compared wire restraining cages. However, it is possible that during the first 60 min of the control period there might have been considerable temperature variations within the free-moving animals as they became used to their new surroundings. Because measurements were not consistently taken during that period, this cannot be ruled out. Even if this were the case, however, all baseline temperature calculations were only taken from the final 30 min of the control period, during which most temperature measurements were quite stable over time.

The mechanism by which the three agents used in this study alter skin temperature have similarities as well as differences. The mechanism by which angiotensin II produces tail skin temperature surges is not clear. It has been suggested (Fregly, personal communication) that this effect may be centrally mediated, for instance, that AII receptors in the central nervous system may be required for this effect to occur. Angiotensin II's ability to induce a tail skin temperature increase has already been linked to the AT1 receptor subtype (8). Administration of both the nonspecific AII antagonist saralasin and the AT1 receptor antagonist losartan have been shown to eliminate the TST rise following AII administration (8).

Isoproterenol, an adrenergic agonist, may also produce skin temperature surges, at least in part, through this AII-mediated pathway by activating the renin-angiotensin system (25). However, it is more likely to act through β-adrenergic receptors to vasodilate the tail skin vascular bed (11,22) either directly or secondary to an increase in metabolic rate in the animals (7). The extent to which each possible mechanism predominates in this effect has not yet been fully elucidated. Because the time course for the response appears longer for isoproterenol compared to AII, it would suggest the mechanism are different for the two agents.

The lack of any decrease in core temperature following isoproterenol treatment was initially surprising because another study using this agent had demonstrated a small transient increase followed by a small, but significant, decrease (7). However, because this reduction in core temperature did not manifest until well after 60 min of treatment, and we stopped measuring temperatures after 60 min postinjection (i.e., after 2-1/2 h in restraining cages), it is possible that a subsequent decrease in core temperature occurred in this study as well.

These temperature responses may be dose related. The authors used the same doses of isoproterenol, angiotensin II, and naloxone that have been used in earlier studies (12–19, 25). It is possible that because the doses used in this study may not have been maximal, some restraint effect may have been missed that would have been demonstrated with different doses of these agents. It is also possible that habituation to restraint stress took place, because by the time core temperatures were measured in Experiment 1 every rat had already been exposed to both restraint and cardboard boxes at least once. If this habituation took place, however, it was with both environments (free moving and restrained). It also did not appear to influence baseline temperatures, because the basal core temperatures of rats prior to morphine tolerance induction (Experiment 3, 36.2°C) appear to be about the same as those in Experiment 1 (36.5 and 36.0°C). Therefore, because our hypothesis was that basal core temperatures would increase rather than remain the same as a result of restraint, and they didn't in either Experiments 1 or 3, the main conclusion—that restraint does not appear to have any general detrimental effect on the ability of pharmacological agents to decrease core temperatures in the Sprague–Dawley rat—remains sound.

In morphine-dependent animals, we demonstrated an increase in baseline core temperatures in this study independent of whether rats were restrained or not. This baseline data is consisted with and validates data from previous studies using restrained animals (16) by showing that the increased core temperatures following morphine tolerance was in fact due to morphine. The increase in basal core temperature by opioids may be due to a resetting of the central thermostat, as previously hypothesized by Cox (4). Naloxone, which also acts centrally (18), yielded results on both skin and core temperatures consistent with and acute regulatory response to a set point suddenly reduced towards normal, that being a surge in skin temperature and a fall in core temperature. As with baseline core temperatures, restraint did not affect the magnitude of either response.

Because naloxone does not change either skin or core temperatures in naive animals (12,17,23), it appears likely that the effects of the opioid antagonist are direct instead of indirect. Aspects of acute morphine withdrawal that are peripherally mediated are susceptible to modulation, however. For example, elimination of both cortical and medullary hormone release via adrenalectomy has been shown to attenuate the increase in skin temperature associated with opioid withdrawal (16). Another study has shown that epinephrine release from the adrenal medulla occurs following naloxone administration to morphine-dependent animals (2). Administration of the α_2-adrenergic agonists clonidine and ST-91 attenuated both the tail skin temperature increase and core temperature decrease associated with this model (15). The same study showed that propranolol, a β-adrenergic antagonist, also attenuated naloxone-induced increases in tail skin temperatures. The possibility therefore remains that at least part of the action of naloxone is to produce the catecholamine surge associated with acute morphine withdrawal, which in turn, may result in activation of the renin-angiotensin system and ultimately an angiotensin II-mediated skin temperature surge. How great a role this indirect pathway may play on skin temperature regulation remains unknown.

In evaluating the time course of the responses to the pharmacological agents, AII appears to be the most rapid, followed closely by naloxone with isoproterenol producing the most delayed response of the three. These time courses are compatible with a possible common role for AII in the response: the direct effector (AII) induces temperature changes before the potential indirect effectors (naloxone and/or isoproterenol). However, this is less likely than the possibility that the three agents have effects independent of one another.

The lack of differences in response to restraint stress that we observed in Sprague–Dawley rats is in contrast to other strains (and depending on the strain, suppliers) of rats, which show more variability in their stress responses to restraint (1,10,21). However, most of our laboratory's prior thermoregulation studies used the same rat strain and supplier as was

used in this study. It is, therefore, likely that if restraint did not influence results in this study it did not influence those in previous studies in this laboratory either.

The results of this study demonstrate that with three different but potentially related models of chemically induced increases in skin temperature, restraint did not decrease the maximal responsiveness of these agents. The results suggest that provided an initial acclimation period is utilized, restraint need not necessarily be considered an interfering factor in similar studies in rats. The results also validated previous studies utilizing pharmacological agents in restrained rats.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dr. C. C. Barney with the use of the Mini-Mitter system. This work was supported by NIH Grant HD18133.

REFERENCES

1. Berkey, D. L.; Meeuwsen, K. W.; Barney, C. C. Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. Am. J. Physiol. 258:R743–R749; 1990.
2. Chang, A. P.-L.; Dixon, W. R. Role of plasma catecholamine in eliciting cardiovascular changes seen during naloxone-precipitated withdrawal in conscious, unrestrained morphine-dependent rats. J. Pharmacol. Exp. Ther. 254:857–863; 1990.
3. Cicero, T. C.; Meyer, E. R. Morphine pellet implantation in rats: Quantitative assessment of tolerance and dependence. J. Pharmacol. Exp. Ther. 184:404–408; 1973.
4. Cox, B.; Ary, M.; Chesarek, W.; Lomax, P. Morphine hyperthermia in the rat: An action on the central thermostats. Eur. J. Pharmacol. 36:33–39; 1976.
5. Dafters, R.; Taggart, P. Biotelemetric investigation of morphine's thermic and kinetic effects in rats. Psychopharmacology (Berlin) 106:195–201; 1992.
6. Dilsaver, S. C.; Overstreet, D. H.; Peck, J. A. Measurement of temperature in the rat by rectal probe and telemetry yields compatible results. Pharmacol. Biochem. Behav. 42:549–552; 1992.
7. Fregly, M. J.; Barney, C. C.; Kelleher, D. L.; Katovich, M. J.; Tyler, P. E. Temporal relationship between the increase in metabolic rate and tail-skin temperature following administration of isoproterenol to rats. In: Cox, B.; Lomax, P.; Milton, A. S.; Schonbaum, E., eds. Thermoregulatory mechanisms and their therapeutic implications. Basel: Karger; 1980:12–18.
8. Fregly, M. J.; Rowland, N. E. Effect of losartan potassium and deoxycorticosterone acetate on tail skin temperature response to acute administration of angiotensin II. Pharmacol. Biochem. Behav. 43:229–233; 1992.
9. Harrison, J.; Turner, P. Comparison of propranolol and I.C.I. 50,172 on isoprenaline-induced increase in skin temperature in man. Br. J. Pharmacol. 36:177–178; 1969.
10. Herbert, J.; Howes, S. R. Interactions between corticotropin-releasing factor and endogenous opiates on the cardioaccelerator, hypothermic, and corticoid responses to restraint in the rat. Peptides 14:145–152; 1993.
11. Katovich, M. J.; Barney, C. C.; Fregly, M. J. Metabolic responsiveness of spontaneously hypertensive rat to isoproterenol. Am. J. Physiol. 241:H497–H504; 1981.
12. Katovich, M. J.; Barney, C. C.; Larsen, E. Effects of peripheral administration of naloxone on B-adrenergic mediated responses. Pharmacology 41:167–176; 1990.
13. Katovich, M. J.; Marks, K. S.; Sninsky, C. A. Effect of insulin in the altered thyroid function and adrenergic responsiveness in the diabetic rat. Can. J. Physiol. Pharmacol. 71:568–575; 1993.
14. Katovich, M. J.; O'Meara, J. The effects of chronic estrogen on the skin temperature response to naloxone in morphine-dependent rats. Can J. Physiol Pharmacol. 65:563–567; 1987.
15. Katovich, M. J.; Pitman, D. L.; Barney, C. C. Mechanisms mediating the thermal response to morphine withdrawal in rats. Proc. Soc. Exp. Biol. Med. 193:129–135; 1990.
16. Katovich, M. J.; Pitman, D.; Schectman, O. Role of the adrenal gland in the thermal response to morphine withdrawal in rat. Can. J. Physiol. Pharmacol. 70:1090–1095; 1992.
17. Katovich, M. J.; Simpkins, J. W.; Berglund, L. A.; O'Meara, J. Regional skin temperature changes in a rat model for the menopausal hot flush. Maturitas 8:67–76; 1986.
18. Katovich, M. J.; Simpkins, J. W.; O'Meara, J. Effects of opioid antagonists and their quaternary analogs on temperature changes in morphine-dependent rats. Life Sci. 39:1845–1854, 1986.
19. Katovich, M. J.; Simpkins, J. W.; O'Meara, J. Effects of acute central LH-RH administration on the skin temperature response in morphine-dependent rats. Brain Res. 494:85–94; 1989.
20. McDougal, J. N.; Marques, P. R.; Burks, T. F. Restraint alters the thermic response to morphine by postural interference. Pharmacol. Biochem. Behav. 18:495–499; 1983.
21. Morley, R. M.; Conn, C. A.; Kluger, M. J.; Vander, A. J. Temperature regulation in biotelemetered spontaneously hypertensive rats. Am. J. Physiol. 258:R1064–R1069; 1990.
22. Rand, R. P.; Burton, A. C.; Ing, T. The tail of the rat in temperature regulation and acclimatization. Can. J. Physiol. Pharmacol. 43: 257–267; 1965.
23. Simpkins, J. W.; Katovich, M. J.; Song, I.-C. Similarities between morphine withdrawal in the rat and the menopausal hot flush. Life Sci. 32:1957–1966; 1983.
24. Wei, E.; Loh, H. H.; Way, E. L. Quantitative aspects of precipitated abstinence in morphine-dependent rats. J. Pharmacol. Exp. Ther. 184:398–403; 1973.
25. Wilson, K. M.; Fregly, M. J. Angiotensin II-induced hypothermia in rats. J. Appl. Physiol. 58:534–543; 1985.