Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 4, pp. 881–885, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0042-G

Food Deprivation Does Not Influence Body or Selection Temperature in Rats Receiving Intraventricular Bombesin[1]

BRIAN S. STUMP, ANDREW J. BANE AND DAVID D. AVERY[2]

Department of Psychology, Colorado State University, Fort Collins, CO 80523

Received 27 May 1993

STUMP, B. S., A. J. BANE AND D. D. AVERY. *Food deprivation does not influence body or selection temperature in rats receiving intraventricular bombesin.* PHARMACOL BIOCHEM BEHAV **48**(4) 881–885, 1994. – Bombesin (BBS), a tetradecapeptide, has been found to have potent hypothermic effects when centrally administered. This study was designed to investigate the relationship between food-deprivation and BBS-induced hypothermia in a temperature selection paradigm. Food-deprived and satiated male Sprague–Dawley rats were given intracerebroventricular (ICV) injections of several doses of BBS and control vehicle. Selection temperature data and changes in core body temperature were measured. BBS produced a significant hypothermia and decrease in selection temperatures in all doses but one. No significant differences in body temperature or selection temperatures were found between food-deprived and satiated animals. The results are consistent with the hypothesis that BBS acts centrally to decrease body temperature set point.

Thermoregulation Set point Bombesin Intraventricular Rats Temperature gradient

BOMBESIN (BBS) is a tetradecapeptide first isolated from the skin of the European frog, *Bombina bombina* (1). It has been found in a variety of mammalian tissues, including canine mucosal layers of the duodenum (14), human lung tissue and blood serum (16,22), and porcine and rat brain tissue (12). Bombesin-like immunoreactivity appears in the hypothalamus (14), and BBS has been found to bind preferentially to synaptosomal fractions (12), suggesting it may act as a neurotransmitter or neuromodulator in the regulation of homeostatic functions.

Previously, central injections of BBS have been found to produce hypothermia in rats, under conditions of food deprivation (2), insulin pretreatment (4), cold exposure (7), and in rats with ventromedial hypothalamic lesions (5). These physiological effects of BBS suggest that it works within the central nervous system to lower the set-point (*Tset*) of body temperature (17). BBS has also been shown to inhibit cold induced thyroid-stimulating hormone release (8), to reduce molecular oxygen consumption (23), to produce cutaneous vasodilation (9), to inhibit cold-induced brown fat thermogenesis (6), and to produce profound hypophagia (13). Behavioral evidence for the reduction of *Tset* has been obtained as well. Avery, Hawkins, and Wunder (1981) reported that heat-stressed rats increased their rate of bar pressing for cold reinforcement. However, research conducted in our laboratory, suggested that the increased response rates for cold reinforcement, in heat stressed rats, and heat reinforcement, in cold stressed animals, may have been caused by hyperactivity resulting from central administration of BBS; and the increases in locomotor activity and increased response for heat reinforcement in the cold were abolished by adrenalectomy (10). Another interpretation of these results may be that adrenalectomy produced sufficient lethargy to impede performance on operant responding. The methodology presented here is an attempt to uncouple the effects of BBS on activity and its impact on motivation to regulate body temperature.

In view of these and other results obtained by Tache, Pittman, and Brown (1980), it has been proposed that BBS merely disrupts normal thermoregulatory control and renders animals poikilothermic (21). The basis of this hypothesis rests, in part, on previous research indicating BBS-induced changes in body temperature (T_b) are only a function of ambient temperature

[1] These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference held in Clearwater Beach, FL, April 22–25, 1993.

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

(21). These authors reported that rats injected with BBS and exposed to temperatures above those of thermoneutrality raised their T_b and lowered their T_b when exposed to temperatures lower than thermoneutrality. In addition, intraventricular injections (ICV) of BBS have resulted in inconsistent hypothermic effects across species. Rabbits show random changes in T_b in response to BBS (11), and goats show a decline in T_b in a thermoneutral environment (9).

It has been shown that food-deprived rats display BBS-induced hypothermia, and food-satiated animals do not display hypothermia in a thermoneutral environment (2). Recently, Barton and Hawkins (1992) found that rats with bilateral VMH lesions will also display BBS-induced hypothermia in a thermoneutral environment, suggesting that some event(s) associated with VMH lesions, hyperphagia, heightened serum insulin levels, and alterations in nutrient metabolism, act as a permissive factor in the production of BBS-induced hypothermia in a thermoneutral environment. This may indicate that BBS-induced hypothermia and/or temperature preference may be dependent on the metabolic states associated with food deprivation.

The objective of this study was to investigate whether food deprivation systematically alters BBS-induced changes in body temperature and temperature selection in rats. If the thermoregulatory effects of BBS are unrelated to the metabolic demands placed on the animal by food deprivation as Hawkins and Barton (1992) propose, then temperature selection and T_b should not be systematically altered by food deprivation. Alternatively, if the metabolic event(s) altered by food deprivation act as a permissive factor for BBS-induced hypothermia, then animals may select different temperatures and have different T_b under food-deprived conditions than when free feeding.

METHOD

Subjects

The subjects were 16 male Sprague–Dawley rats weighing between 250 and 300 g at the beginning of the experiment. The animals were individually housed in hanging rodent cages in a room having a constant ambient temperature of 22 ± 1°C. Lighting was cycled LD 12 L : 12 D. All testing occurred in the dark. Food and water were available ad lib except when noted.

Surgery

Each animal was anesthetized with ketamine-HCl : Rompun (66.0 : 3.3 mg/kg body weight, IM). Chronic indwelling canulae, constructed from 22-gauge hypodermic tubing, were stereotaxically placed within the lateral ventricles, with the incisor bar at 5.0 mm above the interaural line (0.0 mm anterior, 1.2 mm lateral from bregma, and 4.0 mm ventral to the dorsal surface of the calvarium) (15). All animals received at least 1 week of recovery following surgery before testing began.

Peptide Preparation

Bombesin (Sigma) was diluted in H_2O_{di} to 0.1 μg/μl and dispensed in 0.1 ml aliquots. Immediately prior to injection, BBS was diluted with the vehicle control, artificial cerebrospinal fluid (CSF), that was filtered through a 0.2 μm sterilizing filter prior to use. The artificial CSF (ph 7.2) contained: 126 mM NaCl, 25 mM Hepes, 6 mM KCl, 1.45 mM $CaCl_2$, 1 mM NaH_2PO_4, and 0.88 mM $MgSO_4$. BBS doses were 0.025, 0.05, 0.1, and 0.2 μg.

Apparatus

Construction of the temperature gradient, similar to the one used by Stinson and Fisher (1953), has been described in detail (20). Briefly, the gradient was constructed from an 8 ft. length of 6 inch diameter PVC tubing fitted with an 8 ft × 4 in × 0.25 in aluminum floor. Packing paper was placed around the PVC tubing. A 1 inch slit was cut in the paper to permit observation. A heating element and solid CO_2 were placed at opposite ends of the flooring and a small fan was placed at the cold end of the gradient to prevent CO_2 from entering the gradient. The warm end of the gradient had a temperature of approximately 43°C, while the cold end of the gradient had an approximate temperature of −10°C. The gradient was divided into 16 sections with a wax pencil. These sections were used to determine the selection temperature of the subject. The temperature of each section was continuously recorded (Beckman Industrial, 461) by 16 thermocouples placed along the floor of the gradient. The positions of the thermocouples were placed according to approximate thermal units of 2.5 ± 0.1°C. The individual sections varied between 10 and 15 cm, except for the sections located at either extreme. Because in pilot studies animals rarely selected these temperatures, these sections were lengthened to 23 cm.

Procedure

The subjects were randomly divided into two groups. Animals in group 1 were placed on an 18-h food deprivation schedule and group 2 subjects were provided food ad lib. Group 1 was allowed 3 days to habituate to the food deprivation schedule. All sessions were conducted between 1900 h and 0400 h (MST). Baseline measurements were taken until there were no significant differences in selection temperature and core body temperature across baseline sessions. Baseline data were analyzed via dependent *t*-test procedures.

All animals received all doses of BBS and the control injection. All injections were 1.0 μl in volume and were given over a 1-min period. The injection cannulae were left in place for 30 s following the injection to allow the fluid to diffuse away from the cannula tip. Prior to test sessions, each animal from the food deprivation group was matched by baseline selection temperatures with an animal from the nonfood deprived group. During the test phase, animals in matched pairs received the same doses and were tested at approximately the same time of day. Dose order was randomized across treatment sessions and pairs. Treatments were separated by at least 2 recovery days.

Following injections, animals were placed in the warm end of the temperature gradient. After placement in the gradient, 5 min were allowed for the animals to select an ambient temperature and to allow for recovery from any effects of handling. Temperature selection was determined by noting the midpoint of the ventral abdomen and was recorded in reference to the position of a thermocouple. Temperatures selected were recorded at 5, 10, 15, 20, 25, and 30 min. After the final selection, the temperature selected was recorded and the animal was removed from the cold end of the gradient. Core body temperature was measured just prior to injections and immediately after removal from the gradient by inserting a thermistor (YSI 402, Yellow Springs Instrument Co., Yellow Springs, MD) 6.5 cm beyond the anal orifice and recorded from a YSI46 TUE telethermometer.

Histology

All animals were injected (ICV) with cresyl-violet stain, sacrificed by halothane overdose, and intracardially perfused

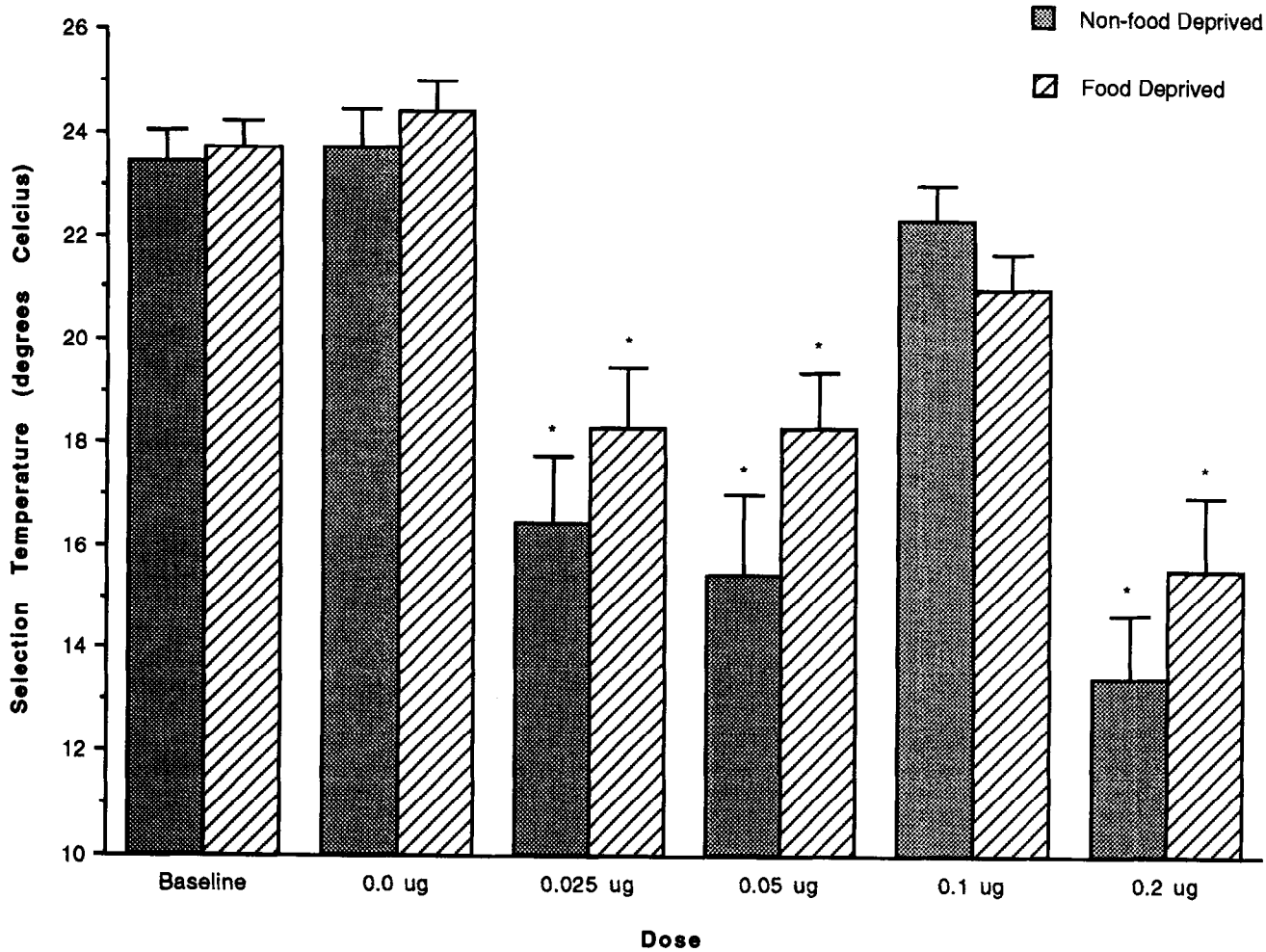

FIG. 1. Mean (±SEM) ambient temperatures selected over the 30-min test session in the thermal gradient apparatus during baseline condition, and after injections of vehicle control (CSF) and 0.025, 0.05, 0.1, and 0.2 μg of bombesin in food-deprived and food satiated animals. *$p < 0.05$ compared with control vehicle (CSF) and baseline ($n = 5$).

with 0.9% saline and 10% formalin solutions. The brains were removed and stored in formalin for 24 h. Frozen 40 μm sections were taken and compared with a standard stereotaxic atlas (15).

Data Analysis

The temperature selection and rectal temperature data were analyzed separately by within-subjects ANOVA. Differences in means were determined by post hoc Newman-Keuls procedures.

RESULTS

Histology

The placement of 10 of the 16 cannulae tips were found to be located within the lateral ventricles. One cannula, from group 1, and two cannulae, from group 2, were not found to be within the lateral ventricles. The data generated by these animals were excluded from statistical analysis. During baseline, the cannulae of three animals became detached; histological analysis was not performed on these animals. Therefore, the statistical analyses included data from five pairs of animals.

Temperature Selection

The main effect of temperature selection was significant, $F(5, 20) = 15.99$, $p < 0.01$. Newman-Keuls procedures revealed that all doses, except 0.1 μg of BBS, produced significantly lower selected temperatures than those selected after control injections (Fig. 1). Over the 30-min session, the average difference in selection temperature between BBS conditions and control conditions ranged between −2.42°C at the 0.1 μg dose and −9.57°C at the 0.2 μg dose.

There were no significant differences among selected temperatures produced by 0.025 μg, 0.05 μg, and 0.2 μg of BBS. Furthermore, no significant differences were found between control and baseline measures of selected temperatures.

Analysis of the effects of food deprivation (0 and 18 h) on selection temperatures disclosed no significant effect, $F(1,4) = 1.93$, $p > 0.05$. The average difference in selection temper-

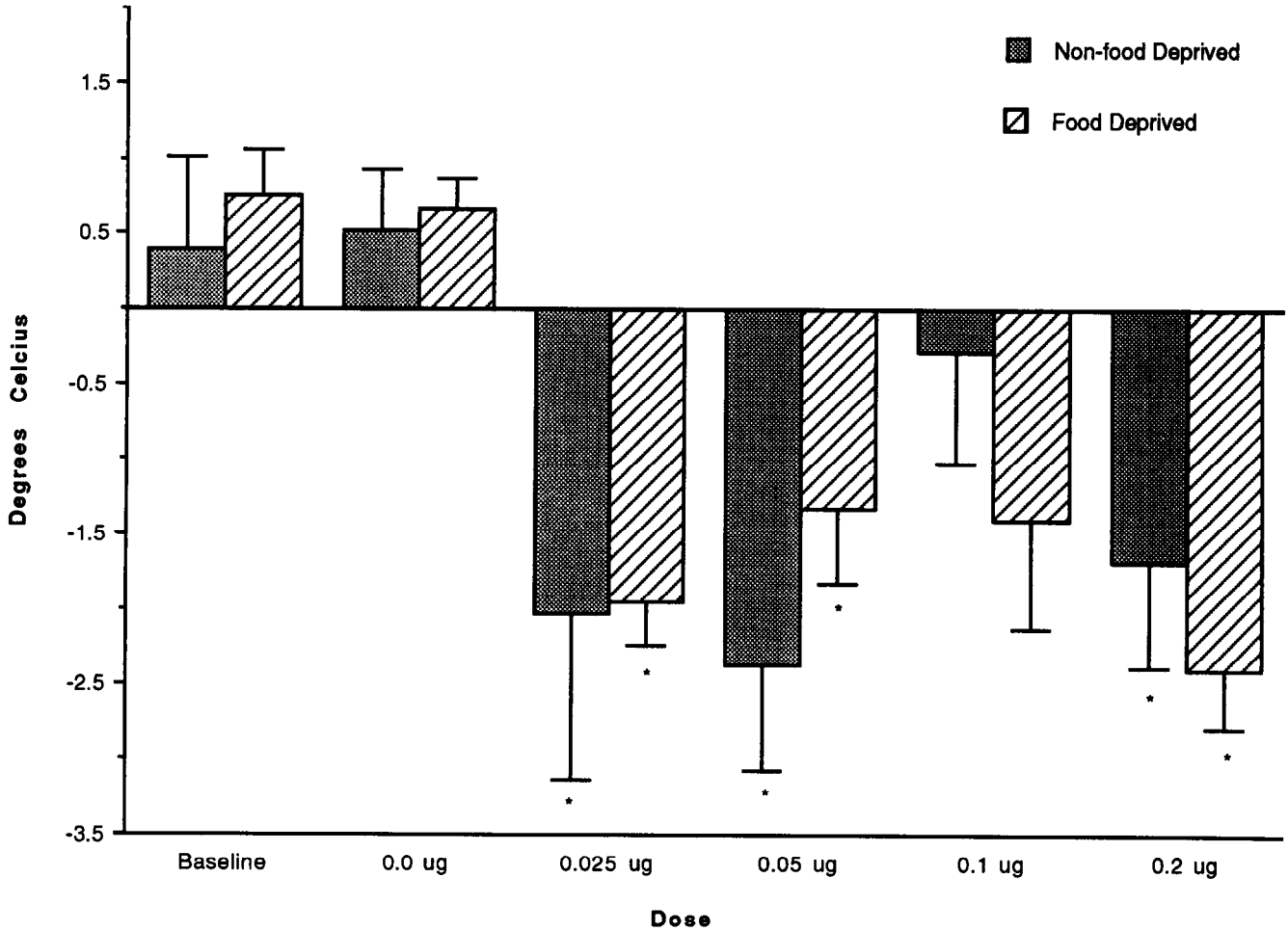

FIG. 2. Mean (± SEM) change from presession to postsession core body temperatures during baseline condition, and after injections of vehicle control (CSF) and 0.025, 0.05, 0.1, and 0.2 μg of bombesin in food-deprived and food-satiated animals. *$p < 0.05$ compared with control vehicle (CSF) and baseline ($n = 5$).

atures between food-deprived animals and nonfood-deprived animals across all conditions was 1.04°C. Additionally, there were no significant differences in selection temperatures among time intervals (5, 10, 15, 20, 25, 30 min), $F(5, 20) = 1.34, p > 0.05$.

Examination of the interactions between: time interval by dose, time interval by food deprivation, dose by food deprivation, and time interval by dose by food deprivation revealed no significant differences.

Core Body Temperature

The mean (± SEM) differences in presession minus postsession core body temperature are presented in Fig. 2. Changes in core body temperature were not significantly different between control and baseline conditions. In addition, food deprivation did not have a significant effect in the change of presession and postsession rectal temperature, $p > 0.05$. BBS, however, did produce a significant hypothermia, $F(5, 20) = 16.34, p > 0.01$. Post hoc tests revealed that BBS produced a significant difference between presession and postsession rectal temperatures at doses of 0.025 μg, 0.05 μg, and 0.2 μg, $p < 0.05$. These effects paralleled the selection temperature measures.

DISCUSSION

The purpose of this study was to explore BBS-induced hypothermia and its relationship to food deprivation. The possibility of an interrelationship is based on several findings: a) BBS-induced hypothermia is not displayed in hunger satiated, noncold-exposed animals (14); b) BBS lowers selection temperatures and, concurrently, decreases core body temperatures in satsatiated animals in comparison to controls after ICV injections (19); and c) VMH lesions permit BBS-induced hypothermia to be expressed under thermoneutral conditions (5).

The present results do not indicate a differential effect of ICV BBS on selection temperatures and core body temperatures in food-satiated vs. food-deprived animals. Consistent with prior results (19), however, the data support the notion that BBS acts centrally to alter T_{set}. Three of the four doses of BBS were shown to decrease significantly both temperature selection and core body temperature. Contrary to these results, in an earlier experiment we found only significant hypo-

thermia at the 0.2 μg dose of BBS (19). The reason for this discrepancy is unknown but, may be due to the influence of the animals' circadian rhythms. During the previous study, test sessions were conducted during the light phase of the light cycle (between 0800 h and 1630 h (MST)) and testing was conducted during the dark phase of the light cycle (between 1900 h and 0400 h (MST)) in the current study. Even so, the fact that the changes in both selection temperature and T_b were very similar for both food-satiated and food-deprived animals indicates that BBS has a robust effect on the regulation of body temperature.

The failure of BBS to induce hypothermia in food satiated animals in a thermoneutral environment as observed in prior research (2) could be due to the impact that feeding has on an organism's metabolic rate. Ingestion of food increases metabolic rate. The etiology of this increase is unknown, but energy expended to assimilate nutrients may mask bombesin's hypothermic effects by impairing an animal's ability to lower its body temperature. As noted previously, although there were no significant differences between selection temperatures produced by each group (see Fig. 1), there was a slight tendency for the hunger-satiated animals to select lower ambient temperatures than the food-deprived subjects. This may reflect an attempt by the animals to compensate for an increased metabolic rate due to recent food intake.

The results of this experiment suggest the mechanism(s) by which BBS causes hypothermia in rats is(are) different from those that produce satiety. The comparable declines in T_b and selection temperatures induced by BBS between sated and fasting animals indicate that in previous research the observation that BBS-induced hypothermia in sated, noncold-exposed animals did not occur may have been caused by some intervening variable such as sympathetic arousal.

Although the results presented here provide evidence that BBS functions centrally to decrease T_{set}, it remains arguable that central administration of BBS simply disrupts thermoregulation. Further investigation of bombesin's effects on thermoregulatory behavior and autonomic responses may allow for a more definitive answer to this issue.

ACKNOWLEDGEMENTS

These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference held in Clearwater Beach, FL, from April 22-25, 1993.

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