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BRIEF COMMUNICATION

Bombesin-Induced Hypothermia: A Dose–Response and Receptor Antagonist Study

ALEX M. BABCOCK,*[1] DAVID A. BAKER* AND TERRY W. MOODY†

*Department of Psychology, Montana State University, Bozeman, MT 59717
†Department of Biochemistry, George Washington University Medical Center, Washington, DC 20037

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BABCOCK, A. M., D. A. BAKER AND T. W. MOODY. *Bombesin-induced hypothermia: A dose-response and receptor antagonist study.* PHARMACOL BIOCHEM BEHAV **43**(3) 957-960, 1992. – Bombesin infusion into the preoptic area (POA) has previously been shown to induce hypothermia in rats that are food deprived or made hypoglycemic with insulin. The present study evaluated the potency and receptor specificity of this response. Bombesin was microinfused into the POA of food-deprived rats (n = 7) and insulin-pretreated rats (n = 7) at doses of 0, 5, 12, 25, and 50 ng/0.5 μl. Changes in core body temperature (rectal) were assessed at 1 h. Hypothermia was observed under both conditions with doses as low as 5 ng (3.1 pM) as compared to vehicle (0 ng). In a separate study, infusion of the reduced peptide bond analog (Psi^{13,14} Leu^{14})bombesin (2.5 μg) prior to bombesin injection (25 ng) was found to prevent the hypothermic response observed in the bombesin control condition. These data suggest that bombesin is a potent hypothermic agent that interacts with gastrin-releasing peptide receptors localized within the POA region to impact thermoregulation.

Bombesin Hypothermia Hypoglycemia Food deprivation Preoptic area Thermoregulation

BOMBESIN is an amidated tetradecapeptide of amphibian origin (1) that shares immunologic epitopes and a spectrum of biologic activity with mammalian gastrin-releasing peptide (GRP) and neuromedin B (NMB) [13,15-18]. In situ hybridization studies using cRNA probes specific for GRP and NMB indicate a wide heterogeneous distribution of neurons that express these peptides (21). Bombesin-like immunoreactive terminals and binding sites have been identified in a variety of brain regions, including the preoptic area (POA) (16,18,23).

Central administration of bombesin has been shown to alter feeding (2,4,11), carbohydrate metabolism (6,10), and core body temperature (T_b) (2,4,7). The effects of bombesin on thermoregulation are complex; peptide-induced changes in T_b appear to be linked to both the metabolic state of the animal (2-5) and the ambient temperature during testing (7). Microinfusions of bombesin into the POA produce hypothermia in rats that are cold exposed (7,19), but fail to alter T_b in food-sated rats tested at normal ambient temperature (2,7). Food-deprived rats tested at normal ambient temperature also exhibit hypothermia following central bombesin (2). Recently, we reported that infusion of bombesin into the POA produces hypothermia in rats that are food deprived or made hypoglycemic with insulin (2,3). Taken together, these findings suggest that bombesin acts within the POA to affect thermoregulation and that the induction of hypothermia is dependent upon factors associated with the fasting state of the animal, as well as the ambient temperature.

Nanogram doses of bombesin intracisternally have been reported to decrease rectal temperatures (7). However, the potency of this hypothermic response within the POA has not been extensively studied. Pittman et al. (19) reported that bilateral infusion of bombesin into the POA at a dose of 25 ng, but not 10 ng, produced hypothermia in cold-exposed rats. A dose of 100 ng was employed by Wunder and coworkers (22) to demonstrate that bombesin infusion into the POA of cold-exposed rats reduces metabolic rate. Our previous studies, using food-deprived animals, only evaluated POA infusions of bombesin at a dose of 50 ng or greater (4,5). In

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

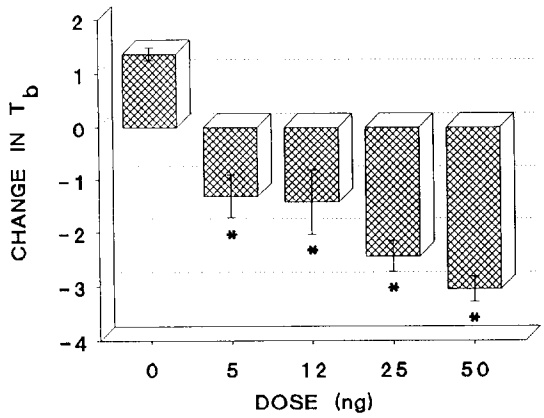

FIG. 1. Mean change (±SEM) in rectal temperature 1 h following POA infusions of bombesin (0, 5, 12, 25, and 50 ng/0.5 μl). Rats (n = 7) were food deprived and tested in a randomized block design. *$p < 0.05$ vs. 0-ng dose.

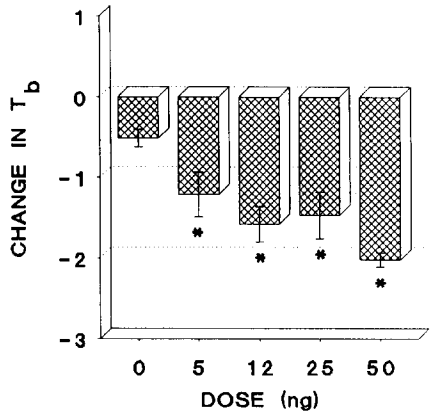

FIG. 2. Mean change (±SEM) in rectal temperature 1 h following infusion of bombesin (0, 5, 12, 25, and 50 ng/0.5 μl) into the POA. Rats (n = 7) were pretreated with insulin (10 U/kg) and tested in a randomized block design. *$p < 0.05$ vs. 0-ng dose.

contrast, others (8) failed to observe hypothermia in food-deprived rats following POA infusion of bombesin at doses that ranged from 25–400 ng. Given this inconsistent finding, and the lack of a systematic evaluation of bombesin potency within the POA, the present study assessed the effects of a range of peptide doses in food-deprived and insulin-pretreated rats. The reduced peptide bond analog (Psi^{13,14}Leu^{14})bombesin has previously been reported to be a potent and specific bombesin receptor antagonist (9,12). The ability of this agent to antagonize bombesin-induced hypothermia in food-deprived rats was also assessed.

METHOD

Animals

Adult, female Sprague-Dawley rats (250–350 g) were used in all experiments. Animals were housed individually in temperature-controlled quarters (23 ± 1°C) and maintained on a 12 : 12 D cycle. Purina rat chow and tapwater were provided ad lib except when noted. Experiments were conducted at 4 h after light onset (1000 h) at an ambient temperature of 23 ± 1°C. A guide cannula constructed of 26-ga stainless-steel tubing (Plastics One, Roanoke, VA) and aimed at the POA

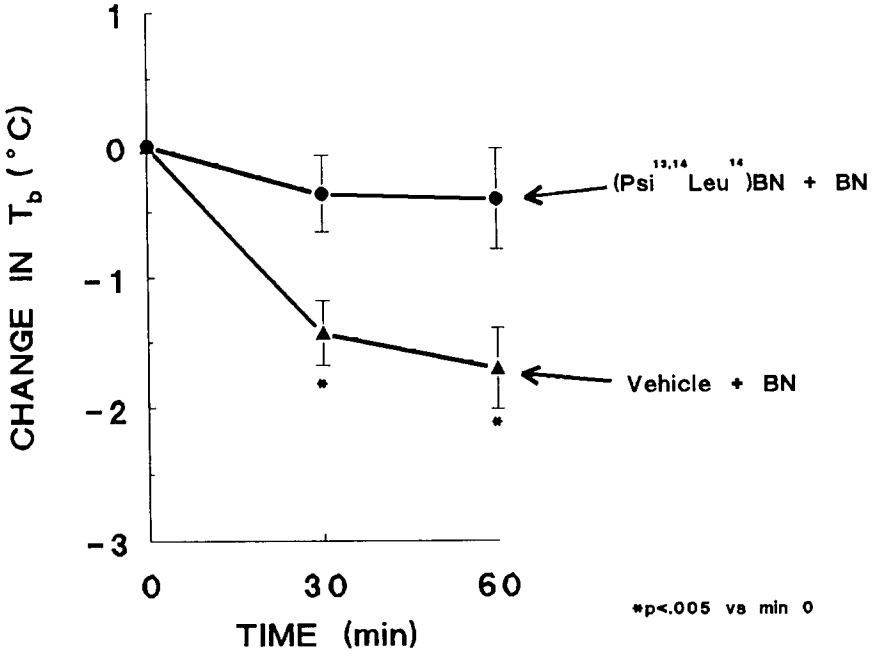

FIG. 3. Mean change (±SEM) in rectal temperature 30 and 60 min following POA infusion of 2.5 μg (Psi^{13,14}Leu^{14})bombesin or antagonist vehicle followed by an infusion of bombesin (0.025 μg). Injections were separated by less than 2 min. Rats (n = 6) were food deprived and treatments were randomized. BN, bombesin.

was implanted into each rat under ketamine HCl (80 mg/kg, IP) and xylazine (5 mg/kg, IP) anesthesia. Cannula assemblies were secured to the skull with anchor screws and dental acrylic. Rats were given at least 5 days to recover prior to any experimentation.

Drugs

Bombesin (Bachem, Tustin, CA) was reconstituted with double distilled water and stored at −20°C. Prior to injection, bombesin was diluted to the proper concentration with sterile 0.9% saline that was previously passed through a 0.2-μm sterilizing filter (Millipore Corp., Milford, MA). The (Psi^{13,14}Leu^{14})bombesin was synthesized using solid phase methodology described previously (9). Central injections were made through a 33-ga cannula designed to extend 1.0 mm beyond the guide cannula tip. Injection volumes were 0.5 μl infused over 30 s using a hand-driven microsyringe. Injection cannulae and tubing were flushed with ethanol and sterile 0.9% saline prior to use. Insulin (Regular Iletin I, Eli Lilly & Co., Indianapolis, IN) was diluted with sterile 0.9% saline (10 U/ml) and administered IM in one study.

Procedure

Fourteen rats were food-deprived for 20 h (n = 7) or injected with insulin (10 U/kg, IM, n = 7) prior to the infusion of various doses of bombesin (0, 5, 12, 25, and 50 ng/0.5 μl) into the POA in a random sequence. Core body temperature was measured to the nearest 0.1°C by inserting a YSI 402 thermistor probe 6.5 cm beyond the anal orifice immediately prior to and at 60 min following injection. The five POA infusions were separated by at least 2 nonexperimental days.

In a separate experiment, food-deprived rats (n = 6) received 2.5 μg of the antagonist (Psi^{13,14}Leu^{14})bombesin or antagonist vehicle followed by an infusion of bombesin (0.025 μg). This ratio of antagonist to peptide is based upon a previous report (14). The infusions were separated by less than 2 min and treatment conditions were randomized.

Histology

At the conclusion of each study, rats were sacrificed with CO_2 and brains were removed and immersion fixed in 10% formalin. Using a vibratome, sections (50 μm) through the region of cannulae invasion were collected and stained with cresyl violet. All rats had injection sites within or on the border of the POA.

Statistical Analysis

Data from the dose-response studies were converted to change scores and evaluated separately using one-way analysis of variance (ANOVA). In the case of significant main effects, the Newman-Keuls procedure was used to assess differences among individual means ($p < 0.05$). For the antagonist study, four planned t-tests were conducted using the Bonferroni correction for multiple a priori comparisons (20). The α level was adjusted to $p < 0.0125$.

RESULTS AND DISCUSSION

The effect of bombesin on core body temperature in food-deprived rats is illustrated in Fig. 1. Reductions in T_b following bombesin ranged from −1.3°C (±0.1; SEM) for the 5-ng dose to −3.1°C (±0.2) for the 50-ng dose. Analysis revealed that the dose of peptide factor was significant, $F(4, 24) = 24.5$, $p < 0.001$. The mean change in temperature following 5, 12, 25, and 50 ng bombesin was significantly different from that of the vehicle condition ($p < 0.05$).

Figure 2 depicts the effect of bombesin in hypoglycemic rats. Reductions in T_b were observed under all conditions ranging from −0.5°C (±0.1) for the vehicle condition to −2.0°C (±0.1) with the 50-ng dose of bombesin. The dose of peptide factor was found to be significant, $F(4, 24) = 6.82$, $p < 0.001$, with subsequent analysis revealing a significant difference between all doses and vehicle ($p < 0.05$).

The effect of (Psi^{13,14}Leu^{14})bombesin on bombesin-induced hypothermia is illustrated in Fig. 3. Infusion of the antagonist vehicle and bombesin resulted in a −1.4 and −1.7°C change in T_b at 30 and 60 min, respectively ($p < 0.01$). Rats infused with (Psi^{13,14}Leu^{14})bombesin prior to bombesin exhibited changes of −0.4°C both at 30 and 60 min that were not found to be significant.

The results of the dose-response studies suggest that bombesin is a potent hypothermic agent when microinfused into the POA. Doses as small a 5 ng (3.1 pM) produced significant reductions in T_b following food deprivation or insulin-induced hypoglycemia as compared to control conditions. Control hypoglycemic rats exhibited a rather pronounced decrease in T_b that was not observed in rats tested under food-deprived conditions. Although we have not observed this response previously using the identical insulin dose (3,4), the fact that rats in the present study were not permitted access to food during testing may have been responsible. Nevertheless, bombesin was found to significantly decrease T_b beyond this condition effect.

The reduced peptide bond analog (Psi^{13,14}Leu^{14})bombesin was found to attenuate bombesin-induced hypothermia. In vitro autoradiographic data indicate that the POA has a moderate density of (^{125}I-Tyr^4)bombesin binding sites. Further, (Psi^{13,14}Leu^{14})bombesin inhibits (^{125}I-Tyr^4)bombesin binding to the POA with an IC_{50} value of 100 nM (T. Moody, unpublished). Because (Psi^{13,14}Leu^{14})bombesin only binds with low affinity to neuromedin B receptors, it would seem that the POA contains predominantly GRP receptors. It is likely that the bombesin-induced hypothermia studied here is mediated by GRP receptors.

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