Pharmacology Biochemistry & Behavior, Vol. 36, pp. 863–867. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Microinfusion of Bombesin Into the Hypothalamic Paraventricular Nucleus Produces Hypothermia in the Insulin-Pretreated Rat

ALEX M. BABCOCK[1] AND CHRIS BARTON

Department of Psychology, University of South Alabama, Mobile, AL 36688

Received 12 March 1990

BABCOCK, A. M. AND C. BARTON. *Microinfusion of bombesin into the hypothalamic paraventricular nucleus produces hypothermia in the insulin-pretreated rat.* PHARMACOL BIOCHEM BEHAV **36**(4) 863–867, 1990.—Bombesin-like peptides are widely distributed in the mammalian central nervous system and participate in the regulation of a variety of autonomic functions. Central injection of bombesin produces hypothermia at normal ambient temperatures, but only if the rat has been food-deprived or made hypoglycemic with insulin. Two experiments were conducted to reevaluate the impact of bombesin microinfusion into the hypothalamic paraventricular nucleus (PVN) on core body temperature and feeding behavior. In Experiment 1, bombesin (0.05 and 0.1 μg/1.0 μl) produced hypothermia, but not hypophagia, in rats (n=5) pretreated with insulin (10 U/kg; IM). Since a similar response was observed in rats with injection sites adjacent to the PVN, a smaller injection volume was evaluated in Experiment 2. Hypothermia, but not hypophagia, was observed in rats (n=5) pretreated with insulin following bombesin (0.025 and 0.05 μg/0.5 μl). Bombesin did not produce hypothermia in rats with injection sites outside of the PVN. These findings suggest that the PVN is a sensitive site for bombesin-induced hypothermia.

Hypoglycemia Thermoregulation Feeding

BOMBESIN is a tetradecapeptide originally isolated from amphibian skin and later detected in the mammalian gastrointestinal tract and nervous system (1,12). Bombesin-like immunoreactivity detected within the CNS appears to be gastrin releasing peptide, which has sequence homology with bombesin (11). The existences of bombesin-like peptides within the hypothalamus, coupled with the wide spectrum of biological activity, suggest a role for these peptides in autonomic and endocrine regulation (12, 14, 15).

Bombesin microinfusion into the lateral cerebral ventricle produces hypothermia at normal ambient temperatures, but only if the rat has been food-deprived (2,3). Evaluation of the neural site(s) which mediate this response have not been extensively studied. In a report by Calisher and Avery (6), microinfusion of bombesin into the hypothalamic paraventricular nucleus (PVN), but not the preoptic area (POA) produced hypothermia in the food-deprived rat. In contrast, we have reported that microinfusion of bombesin into the POA at doses as low as 50 ng produces hypothermia in food-deprived rats, and in rats made hypoglycemic with insulin (4,5). Although bombesin infusion into the PVN has been reported to produce hypothermia, the injection volume used in this study (1.0 μl) does not preclude the possibility that sites adjacent to the PVN are involved. In addition, the authors concluded that the changes were inconsistent suggesting the PVN was not sensitive site for bombesin action (6). Because of these conflicting results and methodological considerations, the present study reevaluated the effect of bombesin microinfusion into the PVN on thermoregulation. Exogenous insulin was used in this study to mimic aspects of the fasted state as previously reported (5). Since the PVN has been implicated in the regulation of feeding behavior (10), and bombesin can produce hypophagia under certain conditions (6,7), changes in food intake were also evaluated.

GENERAL METHOD

Animals

Adult Sprague-Dawley rats (250–350 g) were used in all experiments. Rats were housed individually with 12 hr of light (0700–1900 hr). Purina rat chow and tap water were provided ad lib. Guide cannulae (22-gauge) aimed at the PVN were implanted into each rat under ketamine HCl anesthesia (0.2 mg/g body

[1]Requests for reprints should be addressed to A. M. Babcock, Department of Psychology, University of South Alabama, Mobile, AL 36688.

weight; IM). Cannulae were secured to bone with anchor screws and dental acrylic. A 30-gauge stylet was kept in each guide cannula except during injection.

Drugs

Bombesin (Bachem, Tustin, CA) was reconstituted with distilled water (1 mg/ml), and stored in 20 μg aliquots at −20°C. Immediately prior to use, the peptide was diluted to the proper concentration with a 0.9% saline solution that was previously passed through a 0.2 μm sterilizing filter (Millipore Corp.). Microinjections were made through injection cannulae (28-gauge) which extended 1.0 mm beyond the guide cannula. Injection volumes were 1.0 μl (Experiment 1) or 0.5 μl (Experiment 2) infused over 1 min using a hand-driven microsyringe. Insulin (Regular Iletin I, Lilly) was diluted with 0.9% saline and administered (10 U/ml; IM).

Data Collection

Core body temperature was assessed by inserting a thermistor probe 6 cm beyond the anal orifice. Temperature readings were taken immediately prior to testing, and at 60-min intervals for 2 hr. Food intake was determined by presenting rats with a premeasured amount of standard laboratory chow and weighing the remaining food at 60 and 120 min.

Histology

At the conclusion of each experiment, rats were sacrificed with euthanasia solution (T-61, Taylor Pharmcal Co.) and perfused intracardially with saline (0.9%) followed by a 10% formalin solution. Brains were embedded in paraffin and 15 μm sections were collected through the region of cannulae invasion. Sections were mounted and stained with hematoxylin (9).

Data Analysis

Changes in T_b and food intake were evaluated separately by analysis of variance (ANOVA). Differences between individual means were analyzed using the Dunnetts' procedure. A dependent *t*-test was used to determine whether insulin increased food intake compared to noninsulin controls. Only observed differences at $p<0.05$ were considered significant.

EXPERIMENT 1

The effect of bombesin following PVN microinfusion was evaluated in rats pretreated with insulin. The doses of bombesin and injection volume used were similar to those previously employed to demonstrate bombesin-induced hypothermia in the food-deprived rat (5).

METHOD

Procedure

A randomized block design was used with each rat receiving all treatments in random sequence. Rats (n=7) were weighed and injected with insulin (10 U/kg; IM) or an equal volume of saline. Bombesin (0.025, 0.05, and 0.1 μg/1.0 μl) or peptide vehicle (1.0 μl) were microinfused into the PVN. Food intake and changes in T_b were evaluated for 120 min. Testing began 2 hr following light onset and treatments were separated by at least 3 days.

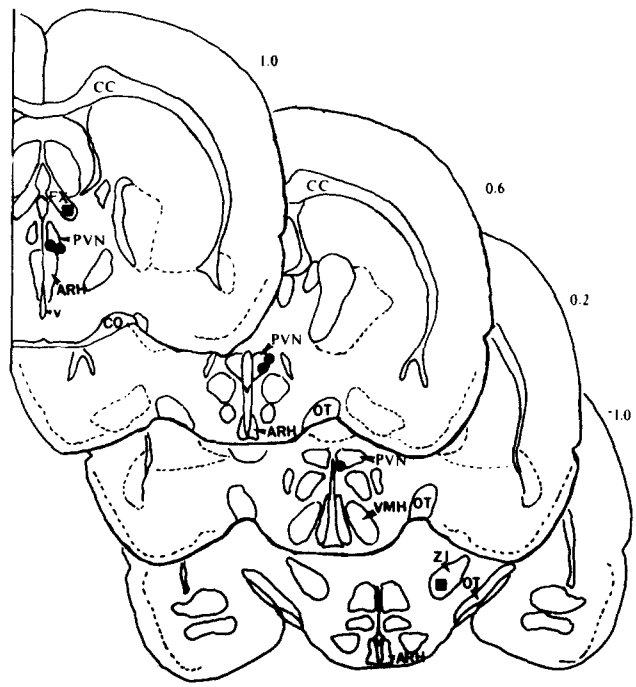

FIG. 1. Injection sites of rats in Experiment 1. Plates are adapted from Pellegrino and Cushman (16) with numbers located to the right of each plate representing the distance from Bregma (mm). Circles depict PVN-positive sites (n=5), while squares represent infusion sites outside of the PVN region (n=2). Abbreviations on plates (also for Fig. 4): PVN, paraventricular nucleus; VMN, ventral medial hypothalamus; ARH, arcuate nucleus; POA, lateral preoptic area; CA, anterior commissure; CO, optic chiasm; CC, corpus callosum; TOL, lateral olfactory tract; OT, optic tract; ZI, zona incerta.

RESULTS AND DISCUSSION

Only data from rats which had injection sites invading the PVN were included in the analysis. A total of 5 rats met this criterion (Fig. 1). Bombesin produced a significant decrease in T_b for insulin-treated rats at 60 min following infusion, $F(3,12)=4.19$, $p=0.03$. Changes observed following the 0.05 and 0.1 μg doses were different from the control condition ($p<0.05$; Fig. 2). Bombesin failed to alter T_b at 120 min, $F(3,12)=0.82$, $p=0.51$. Insulin-pretreated rats with injection sites adjacent to the PVN (n=2) also exhibited a dramatic fall in T_b (−2.2°C and −3.2°C). This lack of site specificity may be related to the large (1.0 μl) injection volumes used (see the General Discussion section). No significant changes in T_b were observed following bombesin in noninsulin pretreated rats at 60 or 120 min following infusion ($p>0.05$; see Table 1).

Insulin significantly increased food intake as compared to noninsulin-treated rats ($p<0.05$). However, bombesin was not shown to significantly decrease food intake under any of the testing conditions at 60 or 120 min ($p>0.05$; Fig. 3).

EXPERIMENT 2

The results of Experiment 1 support the hypothesis that the PVN is a sensitive area for bombesin-induced hypothermia. However, reductions in T_b were also observed following bombesin for regions outside of the PVN. In the present experiment, bombesin was delivered in a smaller injection volume to reduce

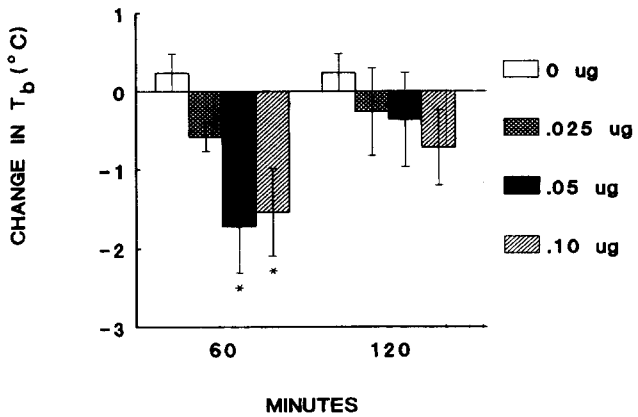

FIG. 2. Mean change in T_b (± standard error of the mean; SEM) at 60 and 120 min following microinfusion of bombesin (0, 0.025, 0.05, 0.10 μg/1.0 μl) into the PVN. Rats (n = 5) were pretreated with insulin (10 U/kg; IM) prior to bombesin administration. $p<0.05$ vs. 0 dose.

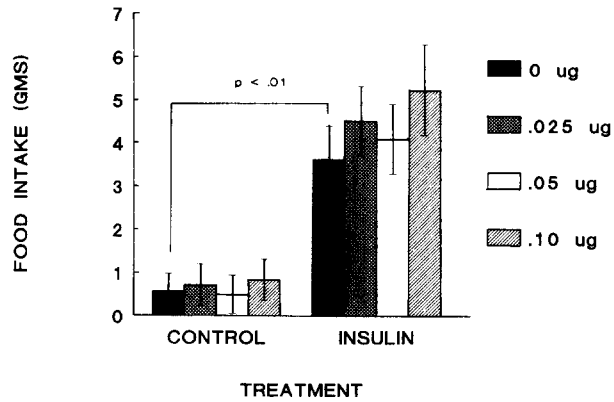

FIG. 3. Mean grams of food intake (± SEM) 2 hr following microinfusion of bombesin (0, 0.025, 0.05, 0.10 μg/1.0 μl) into the PVN. Animals (n = 5) were pretreated with saline (control) or insulin (10 U/kg; IM).

the amount of diffusion. Since the 0.05 μg appeared to produce a maximal reduction in T_b (Experiment 1), a lower range of bombesin doses were evaluated in Experiment 2.

METHOD

Procedures

A total of 10 rats were tested under conditions of insulin pretreatment (10 U/kg; IM) or vehicle (saline; IM). Bombesin was microinfused in a volume of 0.5 μl at doses of 0, 0.01, 0.025, and 0.05 μg. Changes in T_b and food intake were evaluated using methods described in Experiment 1.

RESULTS AND DISCUSSION

Injection sites within the PVN were identified in 5 rats (see Fig. 4). In these animals, bombesin was found to produce hypothermia following insulin pretreatment at 60 min, $F(3,12) = 11.17$, $p<0.001$. The 0.025 and 0.05 μg doses of bombesin resulted in significant changes in T_b compared to controls ($p<0.01$). A significant hypothermia was also observed at 120 min, $F(3,12) = 5.90$, $p<0.01$, with the 0.025 μg bombesin dose being different than control condition ($p<0.05$; see Fig. 5). Hypothermia was not observed in noninsulin-pretreated rats following bombesin at 60 or 120 min ($p>0.05$; see Table 1). In contrast to the results of Experiment 1, bombesin did not produce hypothermia in rats (n = 5) with infusion sites outside of the PVN.

Insulin significantly increased food intake as compared to noninsulin-treated rats ($p<0.05$). However, bombesin did not significantly decrease food intake under any of the testing conditions (Fig. 6).

GENERAL DISCUSSION

In Experiment 1, bombesin produced hypothermia in rats regardless of the injection site. This lack of specificity may be related to the injection volume used since Myers (13) reported that a 1.0 μl droplet can displace approximately 1.1 mm of tissue. Thus, it is possible that bombesin diffused from PVN-negative injection sites into the target area. Alternatively, bombesin may have diffused out of the PVN. To further evaluate this possibility, bombesin was administered in a smaller injection volume (0.5 μl). Significant hypothermia was observed following bombesin in rats pretreated with insulin. Rats with injection sites outside of the PVN did not exhibit hypothermia, which argues for site-specificity. Although diffusion into the 3rd ventricle cannot be ruled out, the present study failed to observe any reduction in feeding behavior which has been previously reported with ICV or POA infusion of bombesin (2,5). Our findings suggest that the PVN is a sensitive region for the impact of bombesin on T_b. The effect of

TABLE 1

MEAN CHANGE (± SEM) IN T_b (°C) AT 60 AND 120 MIN FOLLOWING MICROINFUSION OF BOMBESIN INTO THE PVN OF NONINSULIN PRETREATED RATS (n = 5 FOR EACH EXPERIMENT)

Dose of Bombesin	Experiment 1 (1.0 μl volume)		Experiment 2 (0.5 μl volume)	
	60 min	120 min	60 min	120 min
0	−0.16 ± 0.2	−0.12 ± 0.2	0.52 ± 0.1	0.56 ± 0.2
0.01 μg	N.T.	N.T.	0.48 ± 0.2	0.64 ± 0.3
0.025 μg	0.04 ± 0.3	0.50 ± 0.2	0.54 ± 0.2	0.82 ± 0.4
0.05 μg	−0.64 ± 0.6	0.06 ± 0.5	−0.66 ± 0.7	−0.28 ± 0.6
0.10 μg	0.18 ± 0.3	−0.15 ± 0.3	N.T.	N.T.

N.T. = Not tested.

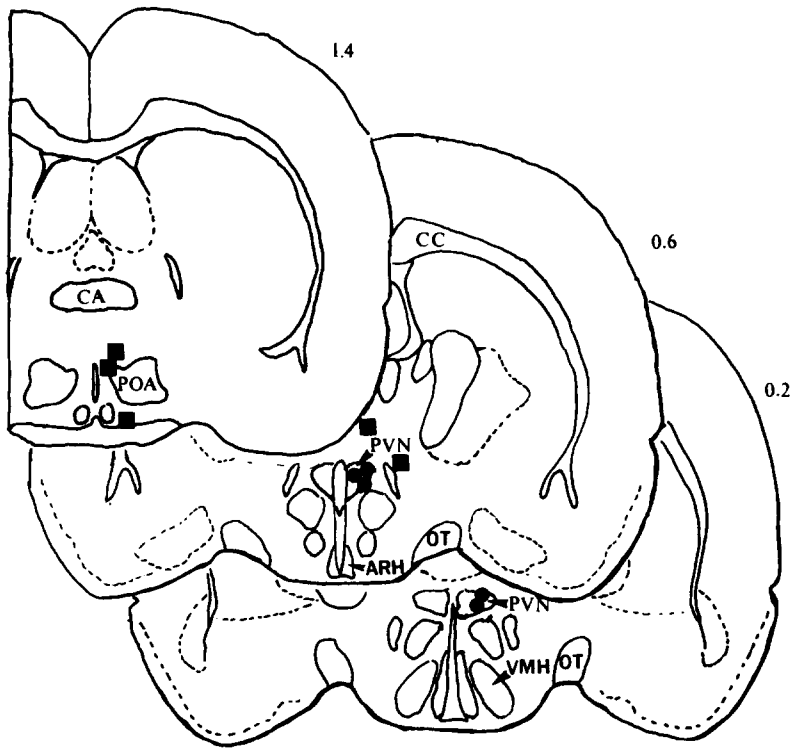

FIG. 4. Microinfusion sites of rats in Experiment 2. Plates are adapted from Pellegrino and Cushman (21) with numbers located to the right of each plate representing the distance from Bregma (mm). Circles depict PVN-positive sites (n = 5), while squares represent infusion site outside of the PVN region (n = 5). See Fig. 1 for abbreviations on plates.

bombesin in the PVN of food-satiated rats has not been previously studied (6). Our findings indicate that microinfusion of bombesin into the PVN does not significantly alter T_b under this condition.

The evaluation of different injection volumes in the present study is important. To our knowledge, only one other study has evaluated the effect of bombesin at this site. This report (6) used an infusion volume that would not preclude the possibility of peptide diffusion to adjacent neural regions. Our data indicate that the PVN is a sensitive site for bombesin-induced hypothermia.

The precise role of the fasted state in bombesin-induced hypothermia is unknown. The ability of bombesin to produce hypothermia in both food-deprived and insulin-pretreated rats suggest that a reduction in the availability of glucose may be a permissive factor. Elevated corticosterone levels associated with food deprivation could also be relevant. It is interesting that

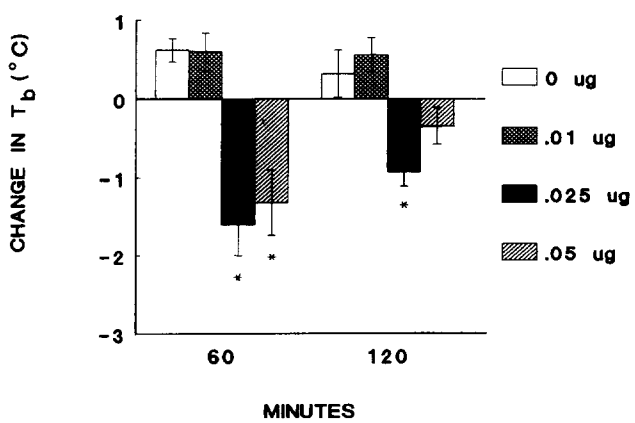

FIG. 5. Mean change in T_b (±SEM) at 60 and 120 min following microinfusion of bombesin (0, 0.01, 0.025, 0.05 μg/0.5 μl) into the PVN. Rats (n = 5) were pretreated with insulin (10 U/kg; IM) prior to bombesin administration. $p<0.05$ vs. 0 dose.

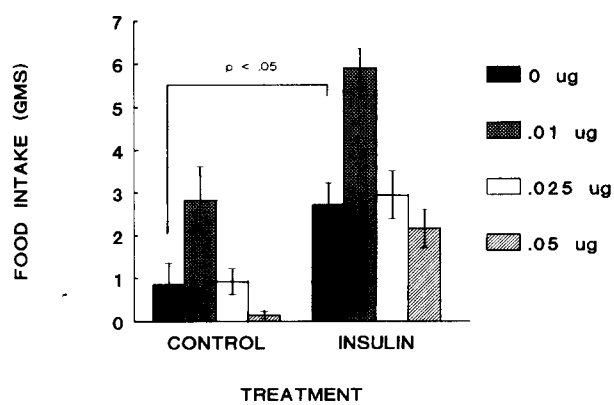

FIG. 6. Mean grams of food intake (±SEM) 2 hr following microinfusion of bombesin (0, 0.01, 0.025, 0.05 μg/0.5 μl) into the PVN. Animals (n = 5) were pretreated with saline (control) or insulin (10 U/kg; IM).

bombesin infusion into the PVN has been recently shown to alter serum metabolic fuel levels (8).

The mechanism of bombesin-induced hypothermia under the present testing condition is not known. Bombesin-like immunoreactive terminal fields localized within the PVN (14,15) could indirectly influence both endocrine and autonomic systems. Cells projecting from the parvocellular subdivision of the PVN terminate in the median eminence while the posterior pituitary receives direct inputs from neurosecretory cells originating from the PVN magnocellular subdivision (18). The parasympathetic cell groups in the motor vagus nucleus and nucleus ambiguus both receive direct inputs from the PVN (17). In addition, the entire sympathetic cell column in the intermediolateral region of the thoracic-lumbar segments of the spinal cord receives direct projections. It is possible that through these descending projections, bombesin could impact the PVN to influence thermoregulation.

The PVN receives inputs from structures which carry information concerning the metabolic state of the animal that could potentially modulate bombesin action. For example, the lamina terminalis and subfornical organs project to the PVN and these circumventricular organs represent regions were blood-born substance can interact with the CNS (18). The PVN also receives a major noradrenergic input from the nucleus of the solitary tract (NTS) and dorsal motor vagus nucleus (17). These nuclei in turn receive inputs from the alimentary tract carried primarily by branches of the facial, glossopharyngeal, and vagus nerves. Future research aimed at determining the origin of bombesin-like immunoreactive terminal fields localized within the PVN will provide important information concerning the role of these peptides in autonomic and endocrine regulation.

ACKNOWLEDGEMENTS

This research was supported by USARC 3-61350 (A.M.B.). We thank Anthony D. Harris for his assistance with data collection.

REFERENCES

1. Anastasi, A.; Erspamer, V.; Bucci, M. Isolation and structure of bombesin and alytesin, two analogous active peptides from the skin of the European amphibians *Bombina* and *Alytes*. Experentia 27:166–167; 1971.
2. Avery, D. D.; Calisher, S. B. The effects of injections of bombesin into the cerebral ventricles on food intake and body temperature in food-deprived rats. Neuropharmacology 21:1059–1063; 1982.
3. Babcock, A. M.; Avery, D. D.; Maitland, K. L. Suppression of operant responding for food and water reinforcement following intraventricular injections of bombesin in the rat. Life Sci. 36: 981–985; 1984.
4. Babcock, A. M.; Barton, C.; Hernon, F.; Perez, L. Bombesin produces hypothermia in insulin-treated rats. Neuropharmacology 28 (4):437–440; 1989.
5. Babcock, A. M.; Barton, C.; Keene, T. Bombesin-induced hypothermia in food-deprived rats. Pharmacol. Biochem. Behav. 34: 539–544; 1989.
6. Calisher, S. B.; Avery, D. D. Injections of bombesin into the substantia nigra produce hypothermia and hypophagia in food-deprived rats. Neuropharmacology 23:1201–1206; 1984.
7. Gibbs, J.; Fauser, D. J.; Rowe, E. A.; Rolls, B. J.; Rolls, E. T.; Maddison, S. P. Bombesin suppresses feeding in rats. Nature 282: 208–210; 1979.
8. Gunion, M. W.; Taché, Y.; Rosenthal, M. J.; Miller, S.; Butler, B.; Zib, B. Bombesin microinfusion into the rat hypothalamic paraventricular nucleus increases blood glucose, free fatty acids and corticosterone. Brain Res. 478:47–58; 1989.
9. Humason, G. L. Animal tissue techniques. 4th ed. New York: W. H. Freeman and Company; 1979.
10. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. 8:163–175; 1984.
11. McDonald, T. J.; Jornvall, H.; Nilsson, G.; Vagne, M.; Ghatei, M.; Bloom, S. R.; Mutt, V. Characterization of a gastrin-releasing peptide from porcine nonantral gastric tissue. Biochem. Biophys. Res. Commun. 90:227–233; 1979.
12. Moody, T. W.; Thoa, N. B.; O'Donohue, T. L.; Pert, C. B. Bombesin-like peptides in rat brain: Localization in synaptosomes and release from hypothalamic slices. Life Sci. 26:1707–1712; 1980.
13. Myers, R. D., ed. Methods in psychobiology. vol. I. New York: Academic Press; 1971:247–280.
14. Palkovits, M. Distribution of neuropeptides in the central nervous system: a review of biochemical mapping studies. Prog. Neurobiol. 23:151–189; 1984.
15. Panula, P.; Yang, H.-Y. T.; Costa, E. Neuronal location of the bombesin-like immunoreactivity in the central nervous system of the rat. Regul. Pept. 4:275–283; 1982.
16. Pellegrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts; 1967.
17. Steffens, A. B.; Scheurink, A. J. W.; Luiten, P. G. M.; Bohus, B. Hypothalamic food intake regulating areas are involved in the homeostasis of blood glucose and plasma FFA levels. Physiol. Behav. 44:581–589; 1988.
18. Swanson, L. W.; Sawchenko, P. E. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. 6:269–324; 1983.