Bombesin-Induced Hypothermia at Normal Ambient Temperatures: Contribution of the Sympathetic Nervous System.

John Christopher Barton
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BOMBESIN-INDUCED HYPOTHERMIA AT NORMAL AMBIENT TEMPERATURES: CONTRIBUTION OF THE SYMPATHETIC NERVOUS SYSTEM

A Dissertation
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
The Department of Psychology

by
John Christopher Barton
B.A., University of South Alabama, 1988
M.S., University of South Alabama, 1990
December 1994
DEDICATION

There are several people to whom this work is dedicated. They have all contributed to my accomplishment of this goal.

First, my loving wife Cheryl. For over six years she has tolerated my status as a student, provided the vast majority of our financial support, moved no less than 7 times and across several states, and has always selflessly given me moral support. Without my wife, there is no doubt in my mind that I couldn’t have completed my efforts to pursue a doctoral degree. For all that she has done I shall be eternally indebted and grateful.

Second, my sweet daughter Erin, who has seen her father as a student for over half of her life. Thank you for all of the understanding during those years when I could not provide you with as much as I would have liked to, both in time and material goods.

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Last, but certainly not least, Dr. Claire D. Advokat. Thank you for your constant interest in my research and your perseverance in my support. You have provided a role
model for me, much in the manner that Dr. Babcock has in the past. You always had time to discuss and debate the scientific issues. You always made me feel that I could accomplish my goals.
ACKNOWLEDGMENTS

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Exceptional recognition goes to Drs. York and Bray for their guidance and fiscal support. They, through their instruction and direction, have greatly shaped my future in research and influenced me to be an analytical thinker. Additionally, they provided the heuristic nucleus for the growth of this project.

Special thanks is given to Mrs. Gail Bley, M.T., of the Clinical Chemistry Laboratory, Pennington Biomedical Research Center for her assistance in assay performance.
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ABSTRACT

Bombesin is a tetradecapeptide first isolated from frog skin. This peptide has potent effects on core body temperature of rats when administered centrally. Bombesin-induced hypothermia at normal ambient temperatures appears to be dependent upon some pre-existent condition which may be related to nutritional status. Four experiments were conducted to examine if the pre-existing condition for bombesin-induced hypothermia at normal ambient temperatures is primarily related to modulation of sympathetic nervous system activation. Experiment I demonstrated that central bombesin resulted in hypothermia in rats tested at normal ambient temperatures under conditions of ad-libitum access to food only when injected peripherally with the ganglionic blocker chlorisondamine. Experiment II demonstrated that bombesin-induced hypothermia in ad-libitum fed rats treated with chlorisondamine is prevented by peripheral injection with the $\beta_3$-adrenergic receptor agonist, CL-316,243. Experiment III demonstrates that bombesin-induced hypothermia seen in acutely food-deprived rats is prevented by peripheral injection of CL-316,243. Experiment IV establishes that bombesin-induced hypothermia seen in acutely food-deprived rats is not dependent upon adrenal catecholamines and that peripheral
corticosterone injection may promote hypothermia, possibly by suppression of corticotrophin-releasing hormone. The results are discussed in relation to sympathetic nervous system modulation and nutritional status.
INTRODUCTION
Isolation and Characterization of Bombesin

A number of peptides that were first isolated in nonneural tissue have also been found in the central nervous system (Krieger, 1983). Many of these neuropeptides appear to function as neurotransmitters and/or neuromodulators (Krieger, 1983). A neurotransmitter is a substance, that when liberated from presynaptic terminals, will interact with receptors on a postsynaptic membrane of an adjacent cell (Krieger, 1983). A neuromodulator represents a substance with nonclassical transmitter actions. It affects the actions of neurotransmitters by modifying their prototypical actions, for example, by blocking the release of specific transmitters or altering the synthesis and degradation of the transmitters (Krieger, 1983).

In 1970, Erspamer and coworkers isolated and characterized the tetradecapeptide bombesin from the skin of the European discoglossid frog Bombina bombina (Erspamer, Erpamer, & Inselvini, 1970). This peptide is structurally similar to the peptide ranatensin (Geller, Govier, Pisano, Tanimura, & Van Clineschmidt, 1970; Van Clineschmidt, Geller, Govier, Pisano, & Tanimura, 1971). Both of these peptides have a general contractile type of activity on the majority of extravascular smooth muscle in mammals (Erspamer, Erpamer, Inselvini, & Negri, 1972).
In 1971 a methodology for the synthesis of bombesin in the laboratory was reported (Bernardi, de Castiglione, Goffredo, & Angelucci, 1971). As research into isolation of bioactive peptides continued several other peptides were discovered that shared similar structure and biological activity with bombesin. In 1970, the isolation of the peptide alytesin from the skin of European discoglossid frog *Alytes obstetricans* was reported (Anastasi, Erspamer, & Bucci, 1971). The amino acid sequence of bombesin and alytesin differ in only two positions, the second and sixth from the N-terminus (Anastasi, Erspamer, & Bucci, 1971). Additionally, the peptide neuromedin-B shares biological activity with bombesin (Minamino, Kangawa, & Matsuo, 1983) as does neuromedin-C (Minamino, Kangawa, & Matsuo, 1984). These peptides, which were discovered to have similar amino acid sequence and share biological activity with bombesin, became known as bombesin-like peptides. The amino acid sequence of several of these bombesin-like peptides is depicted in Figure 1.

Research into the physiological effects of bombesin show that synthetic analogs of bombesin which have modifications of sequence near the N-terminal produce a reduction in receptor binding (Moody, Crawley, & Jensen, 1982). Additional data demonstrated that the C-terminal of the bombesin peptide chain was required for high
<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino Acid Sequence</th>
</tr>
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<tbody>
<tr>
<td>GRP (14-27)</td>
<td>Met-Tyr-Pro-Arg-Gly-Asp-His-Trp-Ala-Val-Gly-His-Leu-Met-NH₂</td>
</tr>
<tr>
<td>Litorin</td>
<td>pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH₂</td>
</tr>
<tr>
<td>Ranatensin</td>
<td>pGlu-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH₂</td>
</tr>
<tr>
<td>Neuromedin-B</td>
<td>Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH₂</td>
</tr>
<tr>
<td>Neuromedin-C</td>
<td>Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH₂</td>
</tr>
</tbody>
</table>

Figure 1. Amino acid sequence of selected bombesin-like peptides.
affinity binding and potent biological activity (Moody, Crawley, & Jensen, 1982). Other researchers have shown that bombesin-like peptides not containing tryptophan appear to have actions specific to the mammalian urinary bladder (Erspamer, 1988). The inclusion of tryptophan in the sequence of bombesin-like peptides is mandatory for the production of its effects on smooth muscle and the gastrointestinal tract (Erspamer, 1988). It was further established that in the bombesin subfamily of peptides, a minimum amino acid chain length of 9 is required for the full spectrum of bombesin-like activity on all type of smooth muscle preparations (Broccardo, Falconieri Erspamer, Melchiorri, Negri, & de Castiglione, 1975). The evidence that bombesin-like peptides with different amino acid structures act on separate tissues of the body suggests the existence of multiple bombesin receptor subtypes distributed in the periphery (Erspamer, 1988).

Gel chromatographic evidence indicates that true amphibian bombesin is not present in mammalian tissues (Walsh, Wong, & Dockray, 1979). In 1979 a mammalian counterpart to bombesin was isolated from porcine gastric tissue (McDonald et al., 1979). This 27-amino acid peptide has an amino acid sequence similar to bombesin, and when administered peripherally, causes the release of gastrin. Thus this new peptide isolated in mammalian tissue became known as gastrin-releasing peptide (GRP).
The carboxyl-terminal sequences of bombesin and GRP are identical in 9 of 10 amino acid residues (Reeve, Cuttitta, Vigna, Shively & Walsh, 1988). Gastrin releasing peptide is considered to be a mammalian analog of amphibian bombesin (Furness, Miller & Costa, 1988). While bombesin exerts biological activities which are similar to those of GRP, and the two are considered analogs, bombesin does not act as a gastrin releasing peptide in amphibians (van Wimersma Greidanus et al., 1984). It has been proposed that amphibian bombesin isolated from the skin of frogs of the Disscoglossid family does not function as a neurotransmitter or neuromodulator in amphibians and that its structural similarity with GRP is secondary to convergent evolution (van Wimersma Greidanus et al., 1984).
DISTRIBUTION OF BOMBESIN-LIKE PEPTIDES

Peripheral Distribution

Bombesin-like immunoreactivity (BLI) in mammalian tissue is found in the greatest concentrations in the stomach (Furness, Miller & Costa, 1988). Significant concentrations of BLI are also found throughout the gastrointestinal tract and minor concentrations are found in the heart and lung (Brown, Allen, Villarreal, Rivier, & Vale, 1978). No detectable concentrations have been found in the adrenals, liver, kidney, pancreas, spleen, or skeletal muscles (Brown, Allen, Villarreal, Rivier, & Vale, 1978). The BLI found in the stomach is localized within nerve cell bodies in the myenteric ganglia, muscle coats, and antral and nonantral regions of the gastric mucosa (Dockray, Vaillant, & Walsh, 1979; Tache, Brown, & Collu, 1979). There were few BLI ganglia found within the submucosa (Christensen & Rick, 1989). In the small intestine, immunoreactive cell bodies are encountered in the myenteric plexus, submucosal plexus, and myenteric ganglia (Dockray, Vaillant, & Walsh, 1979). Immunoreactive ganglia are rare or absent within the mucosa of the small intestine (Furness, Miller & Costa, 1988). Within the large intestine of the rat and mouse, BLI fibers appear in the lamina propria of the mucosa (Moghimzadeh, Ekman, Hakanson, Yanaihara, & Sundler, 1983).
Central Distribution

High concentrations of BLI are found in the hypothalamus and pons/medulla areas (Panula, Yang, & Costa, 1982). Additionally, bombesin-like immunoreactive cell bodies are found in the trigeminal complex and nuclei (Panula, Yang, & Costa, 1982), an area involved in regulation of autonomic functions. Bombesin-like immunoreactive cell bodies have also been found in the preoptic area of the hypothalamus, the ventral and medial parvocellular part of the paraventricular nucleus, and the suprachiasmatic nucleus (Pannula, Nieminen, Falkenberg & Auvinen, 1988).

In the pons/medulla regions, bombesin-like activity has been detected in the laterodorsal tegmental nucleus, the nucleus of the solitary tract, and the parabrachial nucleus (Pannula, Nieminen, Falkenberg & Auvinen, 1988). No detectable concentrations of BLI are found in the regions of the pituitary, pineal, or cerebellum (Brown, Allen, Villarreal, Rivier, & Vale, 1978; Walsh, Wong, & Dockray, 1979).

Using autoradiography, high densities of bombesin-like receptors have been identified in the anterior olfactory nucleus accumbens, and olfactory tubercule (Moody, Getz, O'Donohue, & Rosenstein, 1988). This method also revealed high densities within the central medial and paraventricular nuclei of the thalamus,
the paraventricular nuclei of the hypothalamus, the central and medial amygdaloid nuclei, and the dentate gyrus (Moody, Getz, O'Donohue, & Rosenstein, 1988; Wolf & Moody, 1985). High densities of bombesin-like receptors are also found in the hippocampus (Pert, Moody, Pert, Dewald, & Rivier, 1980).

Moderate densities of receptors have been found in the internal granule layer of the olfactory bulb, frontal cortex of the forebrain, basal caudate putamen, rhinal cortex, rhinal neocortex, septohippocampal nucleus, bed nucleus of the stria terminalis, anterior hypothalamus, and medial preoptic nucleus (Moody, Getz, O'Donohue, & Rosenstein, 1988). The lowest densities were found in the cerebellum, medial forebrain bundle, lateral preoptic nucleus, and ventral pallidum areas of the anterior rat brain (Moody, Getz, O'Donohue, & Rosenstein, 1988). The lateral hypothalamus, and the ventromedial and dorsomedial hypothalamic nuclei also demonstrate low binding densities (Moody, Getz, O'Donohue, & Rosenstein, 1988). Within the midbrain, the central grey and reticular substantia nigra, superficial grey layer of the colliculus, and dorsal raphe nuclei have low densities of bombesin-like activity (Moody, Getz, O'Donohue, & Rosenstein, 1988).
Receptor Subtypes

While GRP, neuromedin-B, and neuromedin-C belong to the same family of bombesin-like peptides, they display significant differences in sequence homology. Neuromedin-C and GRP share the carboxyl-terminal heptapeptide sequence, which is the biologically active segment of these peptides, with amphibian bombesin (Minamino, Kangawa, & Matsuo, 1984; Minamino, Kangawa, & Matsuo, 1983). In contrast, neuromedin-B appears to share a much closer sequence homology with the amphibian oligopeptide litorin (Minamino, Kangawa, & Matsuo, 1984; Minamino, Kangawa, & Matsuo, 1983). These findings have been suggested to constitute a basis for the possible existence of multiple bombesin receptor types (Von Schrenck et al., 1989).

Using autoradiography, it has been shown that two different receptor sites for bombesin-like peptides exist in the periphery (Von Schrenck et al., 1989). Radio-labeled bombesin was found to bind with receptors in both the esophagus and pancreas while radio-labeled neuromedin-B bound only with the epithelial cells of the esophagus (Von Schrenck et al., 1989). In each of these tissue types, binding of the radio-labeled peptides was saturable, dependent on pH, time, and temperature, as well as being reversible and specific (Von Schrenck et al., 1989). In the esophagus the relative potencies for
inhibition of binding of both tracers illustrated that neuromedin-B bound more strongly than bombesin and GRP (Von Schrenck et al., 1989). In the pancreatic tissue assays, bombesin and GRP bound more potently than neuromedin-B (Von Schrenck et al., 1989). Computer analysis of the binding sites revealed that a single binding site existed in assays of each tissue, and that differences in binding between tissues and peptide could only be explained by way of at least two subtypes of bombesin receptors in the periphery (Von Schrenck et al., 1989). There is a heterogeneity of bombesin receptor sites in the rat central nervous system. The rat cerebral cortex appears to contain many more neuromedin-B receptors than bombesin/GRP receptors (Ladenheim, Jensen, Mantey, McHugh, & Moran, 1990). In the nucleus accumbens, neuromedin-B was less effective at inhibiting binding of radio-labeled bombesin, suggesting that this area is more sensitive to binding by bombesin (Ladenheim, Jensen, Mantey, McHugh, & Moran, 1990). In assays of the nucleus of the solitary tract, it appears that neuromedin-B binds with much greater affinity to receptors than does bombesin (Ladenheim, Jensen, Mantey, McHugh, & Moran, 1990).

Using an in vitro autoradiographic methodology, Ladenheim and coworkers have isolated two distinct bombesin receptor subtypes in the rat central nervous system which correspond to bombesin/GRP and neuromedin-B
In examining the differentiation of binding patterns, it was discovered that three general distributions of bombesin-like peptide binding exist. One distinct pattern of binding is referred to as bombesin/GRP-prefering, and is comprised of the supraoptic nucleus, paraventricular nucleus, the arcuate nucleus, and the outer rim of the hippocampal oriens layer (Ladenheim, Jensen, Mantey, & Moran, 1992). A second pattern, which has been designated as neuromedin-B preferring includes the thalamus, dentate gyrus, and ventromedial hypothalamus (Ladenheim, Jensen, Mantey, & Moran, 1992). A third pattern of binding was discovered in the spinal trigeminal tract in the caudal hindbrain, in which both bombesin/GRP preferring and neuromedin-B preferring patterns were found (Ladenheim, Jensen, Mantey, & Moran, 1992).

Examination of regional distributions of bombesin and neuromedin-B demonstrates high densities of neuromedin-B preferring binding sites located in the olfactory bulb and anterior olfactory nucleus, amygdalopryriform transition, amygdalohippocampal area, and lateral amygdala (Ladenheim, Jensen, Mantey, & Moran, 1992). Moderate densities of neuromedin-B binding are found in the ventromedial hypothalamic nucleus, stria terminalis, and medial mammillary nucleus (Ladenheim, Jensen, Mantey, & Moran, 1992). The lowest densities of neuromedin-B
immunoreactivity are found in the infundibular stem, tuber cinereum, and caudate (Ladenheim, Jensen, Mantey, & Moran, 1992). In the thalamic areas, neuromedin-B exhibits high densities in the paraventricular and posteromedian nuclei, moderate densities in the central median, rhomboid, reuniens, and intermediodorsal nuclei (Ladenheim, Jensen, Mantey, & Moran, 1992). Interestingly, there are no thalamic nuclei that contained bombesin/GRP preferring binding sites (Ladenheim, Jensen, Mantey, & Moran, 1992).

In the anterior forebrain regions, bombesin/GRP preferring binding sites are mainly located in the hippocampus, with high densities in the magnocellular region of the paraventricular nucleus, arcuate, suprachiasmatic nuclei, and lateral mammillary bodies (Ladenheim, Jensen, Mantey, & Moran, 1992). Additional high density sites are found in the lateral and ventral aspects of the bed nucleus of the stria terminalis and the outer edge of the hippocampal oreins (Ladenheim, Jensen, Mantey, & Moran, 1992). Moderate densities of bombesin/GRP-preferring sites are located in the molecular layer of the hippocampus, and layers I-VI of the cortex with the greatest densities in the parietal cortex (Ladenheim, Jensen, Mantey, & Moran, 1992).

In the midbrain, moderate levels of bombesin/GRP-preferring densities were found in the reticular subdivision of the substantia nigra, and low
levels of binding in the ventral tegmental region (Ladenheim, Jensen, Mantey, & Moran, 1992). Moderate levels of neuromedin-B preferring binding sites were found in the dorsal raphe, median raphe, and pontine nuclei while the lateral and dorsomedial interpenduncular nucleus exhibited high binding densities (Ladenheim, Jensen, Mantey, & Moran, 1992). Low levels of bombesin/GRP preferring binding densities was found in the ventral tegmental region, while low levels of neuromedin-B preferring densities were identified in the dorsal cortex of the inferior colliculus (Ladenheim, Jensen, Mantey, & Moran, 1992).

In the hindbrain moderate densities of neuromedin-B preferring binding sites were located in the lateral and medial parabrachial nuclei, locus coeruleus, prepositus hypoglossal nuclei, nucleus of the solitary tract, and the periventricular grey of the dorsal rim of the fourth ventricle (Ladenheim, Jensen, Mantey, & Moran, 1992). High densities of bombesin/GRP preferring binding sites were observed in the spinal trigeminal nucleus and nucleus ambiguus, while moderate densities were seen in the gigantocellular reticular nucleus (Ladenheim, Jensen, Mantey, & Moran, 1992). The lowest densities of bombesin/GRP preferring binding sites were observed in the inferior olivary nucleus (Ladenheim, Jensen, Mantey, & Moran, 1992).
Studies performed using bombesin receptor antagonists have demonstrated similar distributions of receptor subtypes in the central nervous system (Ladenheim et al., 1993) and the periphery (Von Schrenck et al., 1990). Clearly there is a wide spread distribution of at least two known bombesin receptor subtypes both in the central nervous and in peripheral tissues, specifically the gastrointestinal tract.
PHYSIOLOGICAL ACTIONS OF BOMBESIN

Central Actions

Effect on the Autonomic Nervous System

Central administration of bombesin-like peptides produces a variety of changes in autonomic nervous system substrata. Injection of bombesin into the intracerebral ventricles (icv) produces a significant increase in plasma levels of norepinephrine and epinephrine in the rat (Brown, Carver & Fisher, 1988). Centrally administered bombesin has a markedly greater potential to induce elevations in plasma epinephrine levels relative to norepinephrine (Brown & Fisher, 1984). Bilateral adrenalectomy abolishes the increase in epinephrine that follows central administration, suggesting that bombesin stimulates brain regions which have neural influence on the adrenal glands (Brown & Fisher, 1984; Brown, Tache, & Fisher, 1979; Fisher & Brown, 1984). The specific brain region/s through which bombesin exerts its influence on norepinephrine levels have not been located, but may be the same site at which it regulates epinephrine levels, which is the rostral aspect of the nucleus of the solitary tract (Brown, Tache, & Fisher, 1979). It is possible that the brain site(s) responsible for this effect may be anterior to, or project neural pathways through, the lateral hypothalamus since lesions of the lateral hypothalamus, or transections lateral or posterior to it
attenuate bombesin-induced elevations of glucose found secondary to adrenal stimulation (Gunion, Grijalva, Tache, & Novin, 1984).

The stimulation bombesin exerts on the adrenal medulla appears to be mediated by neural rather than humoral events (Brown, 1981; Brown & Fisher, 1984; Brown, Tache, & Fisher, 1979; Somiya & Tonoue, 1984). Central injection of bombesin has been demonstrated to increase adrenal epinephrine secretion, inhibit gastric acid secretion, and elevate heart rate and blood pressure via sympathetic nervous system mediation (Brown & Tache, 1981). In addition to adrenal modulation of metabolic fuels, bombesin has been observed to significantly suppress both vagal and sympathetic outflow to the stomach while markedly stimulating activity in the laryngeal branch of the vagus and the adrenal sympathetic nerve (Somiya & Tonoue, 1984).

Bombesin has also been shown to have potent effects on other parameters of autonomic function. Injections of bombesin into the lateral ventricles of rats results in a reduction of the normal rise in heart rate and oxygen consumption secondary to exposure to cold which may be secondary to modification of a central mechanism controlling cardiac function (Fisher, Cave, & Brown, 1985). Additional research in this area illustrates that central administration of bombesin produces elevations in
mean arterial pressure, heart rate, and suppresses cold-induced tachycardia (Fisher & Brown, 1984). Only the elevation in mean arterial pressure seen after bombesin administration is attenuated by adrenalectomy (Fisher & Brown, 1984), suggesting that the other changes seen were secondary to neural rather than humoral mechanisms. Central bombesin has also been found to modulate respiratory function. Bombesin icv produces elevations in minute volumes in rats which are secondary to an overall increase in respiratory rate and tidal volume, as well as significant increases in sighing respiration (Niewoehner, Levine, & Morley, 1983).

Rats treated with bombesin centrally do not exhibit cold-induced accumulation of dopamine in the interscapular brown fat of rats treated with a dopamine β-hydroxylase inhibitor (Brown, Allen, & Fisher, 1987). This finding suggests that bombesin inhibits cold-induced sympathetic outflow to brown fat. This inhibition of dopamine accumulation in brown fat in cold-exposed animals was found to be a viscerotopically specific response, as dopamine levels in other tissues were not effected (Brown, Allen, & Fisher, 1987). Others have reported that animals tested with bombesin at normal ambient temperatures exhibit reduced interscapular brown fat thermogenesis relative to core body temperatures (Shido, Noda, & Nagasaka, 1987).
Effect on the Gut

Central bombesin decreases both volume and concentration of gastric secretions in conscious, pylorus-ligated rats (Tache, Vale, Rivier, & Brown, 1980). This effect is dose dependent, long lasting, and reversible. Bombesin injected into the cisterna magna produces potent increases in mucus secretion from the gastric wall in rats, which is of long duration (Tache, Vale, Rivier, & Brown, 1980). Intraventricular bombesin produces a dose dependent inhibition of gastric emptying and small intestine motility, while producing a transient stimulation of, large intestine motility (Porreca & Burks, 1983; Porreca, Burks, & Koslo, 1985; Tache, Brown, & Collu, 1979) and duodenal contraction frequency (Porreca, Burks, & Koslo, 1985). This effect appears to be mediated by a disruption of normal peristaltic activity, rather than a simple reduction in wave frequency since various areas of the gastrointestinal tract are affected differently. The inhibition of gastric emptying seen in rats receiving central bombesin is abolished by vagotomy (Porreca & Burks, 1983).

A large number of studies have established the potent ability of bombesin to inhibit gastric acid secretion after central administration (Brown & Tache, 1981; Gunion, Tache, Walsh, & Novin, 1984; Tache, 1985; Tache, Lesiege, & Goto, 1986; Tache, Vale, Rivier, & Brown, 1980; Tache,
Vale, Rivier, & Brown, 1981; Yang, Cuttitta, Raybould, & Tache, 1989). Reports indicate that bombesin injected into the lateral ventricles (Brown & Tache, 1981; Gunion, Tache, Walsh, & Novin, 1984; Tache, 1985; Tache, Lesiege, & Goto, 1986; Tache, Vale, Rivier, & Brown, 1980; Tache, Vale, Rivier, & Brown, 1981) or administered intrathecally (Yang, Cuttitta, Raybould, & Tache, 1989), reliably suppresses gastric acid secretion in rats. This reduction in gastric acid secretion results in a general increase in gastric pH, and is not attenuated by abdominal vagotomy (Tache, Lesiege, & Goto, 1986), nor do prostaglandin or opioid pathways mediate this activity (Tache, 1985). Lesions of the ventromedial hypothalamus do not attenuate bombesin-induced reductions in acid secretion (Gunion, Tache, Walsh, & Novin, 1984). Interestingly, intracisternal injection of bombesin produces a long lasting and robust increase in serum gastrin levels (Tache, Marki, Rivier, Vale, & Brown, 1981; Tache, Vale, Rivier, & Brown, 1980). It has been reasoned that bombesin inhibits the acid secretory effect of endogenous gastrin by elevating gastric pH. It has also been suggested that bombesin produces changes in gastric acid secretion by excitation of the sympatho-adrenomedullary system (Okuma, Yokotani, & Osumi, 1987). The exact mechanism by which bombesin influences gastric pH remains unclear.
Endocrine Effects

Central bombesin has been found to modulate the secretion of a wide variety of endocrine products. Bombesin injection into the central nervous system increases serum glucose, free fatty acids, and corticosterone levels (Babcock, Barton, Gunion, & Rosenthal, 1992; Gunion et al., 1989). The ability of central administration of bombesin to induce hyperglycemia has been well established (Brown, Tache, & Fisher, 1979; Gunion, Grijalva, Tache, & Novin, 1984; Brown, 1981). This effect appears to be mediated by bombesin-induced stimulation of epinephrine from the adrenal medulla (Brown, Tache, & Fisher, 1979). Adrenalectomy, but not hypophysectomy prevents bombesin-induced hyperglycemia, further supporting the role of the adrenals in this event (Brown, Tache, & Fisher, 1979). In addition to inducing hyperglycemia, central administration of bombesin results in elevations of serum epinephrine (Brown & Fisher, 1984), hyperglucagonemia, and relative hypoinsulinemia, with insignificant effect on dopamine or norepinephrine (Brown, 1981; Brown, Tache, & Fisher, 1979). Brown has suggested that bombesin-induced changes seen in glucose, glucagon, and insulin levels are secondary to the release of adrenal epinephrine (Brown, 1981). Additionally, it has been demonstrated that lesions of the lateral hypothalamus or transections of the lateral or posterior borders of the
lateral hypothalamus block bombesin-induced hyperglycemia (Gunion, Grijalva, Tache, & Novin, 1984). These findings would suggest that bombesin produces these effects by acting on brain sites which are adjacent to, or project neural efferents through, the lateral hypothalamus.

Bombesin also inhibits stress-induced elevations in prolactin while having no effect on corticosterone, growth hormone, or lutenizing hormone in one study (Tache, Brown, & Collu, 1979), while others have demonstrated that gastrin-releasing peptide suppresses growth hormone secretion (Pinski et al., 1992), and bombesin elevates serum corticosterone levels (Babcock, Barton, Gunion, & Rosenthal, 1992; Gunion et al., 1989). The disparity between these findings may be related to the fact that in the stressed animals these hormone levels could have been maximally stimulated, and thereby resistant to the effects of bombesin.

Peripheral Actions of Bombesin

Effect on Smooth Muscle

When administered peripherally, bombesin has a potent effect on smooth muscle including the intestine, blood vessels, urinary bladder, and uterus (Erspamer, Erpamer, & Inselvini, 1970; Erspamer, Erpamer, Inselvini, & Negri, 1972). These effects vary between species; dogs, cats, rabbits, and rats experience an increase in blood pressure
and smooth muscle tone, while primates experience a
decrease in blood pressure (Erspamer, Erspamer, Inselvini,
& Negri, 1972). Additionally, intravenous administration
of bombesin produces dose-dependent increases in mean
arterial pressure and heart rate in rats (Bayorh &
Feuerstein, 1985).

**Effect on the Gut**

Bombesin injected either subcutaneously or
intravenously stimulates secretion of gastric acid as well
as pepsin from the fundus of denervated stomach in the dog
(Bertaccini, Erspamer, & Impicciatore, 1973). Bombesin
alters gastric motility in rats, dogs, and man. In the
rat, bombesin induces spasmogenic activity independent of
the autonomic nervous system (Bertaccini & Impicciatore,
1975). In man, bombesin exerts a contractile effect on
the antrum and the pylorus while having an inhibitory
effect on motility on the stomach body and the fundus
(Bertaccini & Impicciatore, 1975). While bombesin has
potent effects on the motility of the stomach tissue,
there is no effect of bombesin on gastric emptying
(Hostetler, McHugh, & Moran, 1989). Additionally,
bombesin has been demonstrated to have potent stimulatory
effects on the rat colon (Erspamer, Erpamer, & Inselvini,
1970).
Endocrine Effects

Bombesin has been found to produce several changes in endocrine function when administered peripherally. Intravenous bombesin injections into food-deprived baboons produces an elevation of plasma insulin levels without changing plasma glucose (Woods, Stein, Figlewicz, & Porte, Jr., 1983). When animals are allowed to feed after bombesin injection there is a reduction in plasma insulin after completion of the feeding interval (Woods, Stein, Figlewicz, & Porte, Jr., 1983). In addition, intravenous administration of bombesin increases plasma glucagon levels in the dog (Kaneto, Kaneko, Nakaya, Kajinuma, & Kosaka, 1978; Vaysse et al., 1981) and suppresses the post-prandial rise in serum glucose (Woods, Stein, Figlewicz, & Porte, Jr., 1983).

It appears that bombesin exerts a potent effect on pancreatic tissues. In dogs, systemic administration of bombesin produces an increase in pancreatic polypeptide in a fashion similar to ingestion of a meal of meat (Konturek, Konturek, Bielanski, & Szewczyk, 1989). This increase in pancreatic polypeptide can be blocked by the cholecystokinin antagonist, L-364,718 (Konturek, Konturek, Bielanski, & Szewczyk, 1989). This would suggest that bombesin produces this effect via activation of endogenous cholecystokinin. Treatment of pancreatic tissue with bombesin results in increased amylase release (Lee,
Jensen, & Gardner, 1980), which can be blocked with a bombesin antagonist (Sekar, Uemura, Coy, Hirschowitz, & Dickinson, 1991). These results have been replicated in conscious rats with chronic pancreatic fistula (Jaworek, Konturek, Konturek, Cai, & Schally, 1992). Bombesin stimulates a rapid increase in the concentration of free cytosolic calcium ions which is believed to be, in part, responsible for bombesin effect on stimulation of pancreatic enzymes (Pandol & Mendius, 1988).

In isolated tissue preparations, bombesin has been shown to stimulate somatostatin release as well as gastrin (DuVal et al., 1981). In conscious dogs, intravenous bombesin increases somatostatin-like immunoreactivity, which can be partially attenuated by administration of atropine sulfate (De Graef & Woussen-Colle, 1985) implicating, at least in part, a cholinergic mechanism. The increase in gastrin release by bombesin in isolated tissue (Azuma, Taggart, & Walsh, 1987; DuVal et al., 1981) has also been reproduced in dogs (Bertaccini, Erspamer, Melchiorri, & Sopranzi, 1974). In addition to its other endocrine effects, bombesin has been found to regulate the release of cholecystokinin from the small intestine (Bertaccini, Erspamer, & Impicciatore, 1973) and stimulate the release of prostaglandins from isolated rabbit ear preparations (Juan, Sametz, Petronijevic, & Lembeck, 1984).
Other Effects

Bombesin administered peripherally enhances memory retention in rats (Flood & Morley, 1988). This is attenuated by vagotomy, suggesting that an ascending vagal pathway participates in this event (Flood & Morley, 1988). This finding is similar to those of previous reports which implicate feeding in the enhancement of memory and suggests that this event is not specifically due to feeding, but is reliant on the stimulation of gastrointestinal peptides by food intake. Peripheral bombesin treatment also enhances slow wave sleep (de Saint Hilaire-Kafi, Gibbs, & Nicolaidis, 1989).

Bombesin has been found to produce other changes after peripheral administration. Intraperitoneal injection of bombesin in rats increases metabolism, as measured by computerized open circuit respirometer (Even, de Saint Hilaire, & Nicolaidis, 1991). The existence of bombesin in the periphery has also been linked with several types of neoplasms, and is believed to act as a growth factor in these tissues (Wiedermann, Ruff, & Pert, 1988). Included in these neoplasms are testicular cancer (Fathi et al., 1993), small cell lung carcinoma (Kane et al., 1991; Wiedermann, 1989), and glioblastoma (Wang et al., 1992).
BEHAVIORAL ACTIONS OF BOMBESIN

Effect of Central Bombesin on Feeding

studies, central injections of bombesin have been found to suppress operant responding for both food and water (Babcock, Avery, & Maitland, 1985; Gibbs, Kulkosky, & Smith, 1981; Kulkosky, Gibbs, & Smith, 1982), while in others it was found to be specific to food reward (Avery & Calisher, 1982; Stuckey & Gibbs, 1982). These findings have brought some researchers to the belief that bombesin may act as a potential aversive agent when administered centrally. Kulkosky and colleagues (Kulkosky, Gibbs, & Smith, 1982) reported an increase in grooming after central doses of bombesin which suppress food intake. Yet other behaviors common to feeding, but not specific parameters of consumption, such as chewing, are not altered by intraventricular injections of bombesin (Morley, Levine, Murray, Knelp, & Grace, 1982). Other data would lend additional support to the belief that bombesin does not create an aversive state when administered centrally. Bombesin does not produce a conditioned taste aversion at doses which have been reported to suppress food intake (Kulkosky, Gray, Gibbs, & Smith, 1981). Additionally, low doses of bombesin which suppress feeding induce changes in grooming and locomotion closely related to those seen under conditions of normal satiety (McCoy & Avery, 1990). The most convincing evidence to support the hypothesis that central bombesin may act as a satiety agent comes from findings that very
low doses into the fourth ventricle produce reductions in feeding with no increase in grooming or locomotion (Ladenheim & Ritter, 1988), that increases in bombesin-like peptide activity in the hypothalamus and hippocampus occur after feeding (Kateb & Merali, 1992), and that newly developed bombesin receptor antagonists injected centrally result in increase food intake (Merali, Moody, & Coy, 1993). Thus, evidence exists suggesting that bombesin may mimic a possible satiety action of gastrin-releasing peptide by acting on central receptor sites, and that while having potent anorectic effects in a variety of brain regions, specific changes in behavior related to reductions in feeding and potential satiety may be controlled by periventricular areas. One theory that has been forwarded, is that bombesin acts both within the central nervous system and periphery to integrate events associated with regulation of food intake, development of satiety, and satiety related behaviors (McCoy & Avery, 1990).

**Effect of Peripheral Bombesin on Feeding**

Unlike the case with central bombesin injections, there appears to be a much greater agreement among researchers that peripheral administration of bombesin produces satiety without aversion or malaise (McCoy & Avery, 1990). Peripheral administration of bombesin

Research has revealed a fixed sequence of behaviors which are characteristic of satiety in the rat (Antin, Gibbs, Holt, Young, & Smith, 1975). These behavioral parameters are: Termination of feeding, short term grooming and exploration, followed by rest or sleep (Antin, Gibbs, Holt, Young, & Smith, 1975). Peripheral administration of bombesin elicits a sequence of events associated with normal satiety such as, grooming,
sniffing, locomotion, and rearing, followed by withdrawal from the site of feeding and rest or sleep (Gibbs, 1985; Gibbs, Kulkosky, & Smith, 1981). Additionally, bombesin reduces operant responding for food as a reward (Babcock, Avery, & Maitland, 1985; Flood, Silver, & Morley, 1990), indicating a reduction of hunger. Peripherally administered bombesin is equally potent at suppressing both liquid and solid meals (Gibbs et al., 1979), while not suppressing intake of water (Kulkosky, Gibbs, & Smith, 1982; Kulkosky, Gray, Gibbs, & Smith, 1981). The suppression of feeding observed after peripheral bombesin administration is not due to changes in gastric emptying (Gibbs & Smith, 1986; Hostetler, McHugh, & Moran, 1989), and does not require vagal innervation of the stomach to produce these effects (Gibbs & Smith, 1986; Morley, Levine, Kneip, & Grace, 1982; Smith, Jerome, & Gibbs, 1981). Added evidence for peripheral bombesin as a satiety agent are the findings that it elicits satiety in sham fed rats (Martin & Gibbs, 1980), and that it does not produce a conditioned taste aversion (Kulkosky, Gray, Gibbs, & Smith, 1981). There is additional evidence that suggests bombesin suppresses food intake independent of peripheral release of cholecystokinin (Gibbs & Smith, 1986). While the exact site through which peripheral bombesin produces satiety is unknown, there is data to suggest that it acts on receptors in the gut to modulate
feeding. Bombesin-like immunoreactivity has been well demonstrated in the gastrointestinal tract (Brown, Allen, Villarreal, Rivier, & Vale, 1978). Perfusion of the celiac artery with bombesin produces suppression of feeding more so than perfusion of the superior mesenteric artery, or when injected intraperitoneally (Kirkham, Gibbs, & Smith, 1991). Since the celiac arterial blood supply perfuses the stomach, pancreas, liver, spleen, and proximal duodenum, it would seem likely that one or more of these organs may provide a primary site of bombesin action on feeding in the periphery. While the vagal innervation of the stomach is not necessary for bombesin to produce reduction in feeding, there is evidence for a gut brain axis of action. Complete neural disconnection of the gut from the brain blocks bombesin-induced satiety (Stuckey, Gibbs, & Smith, 1985) and peripheral treatment with capsaicin, a neurotoxin known to destroy small diameter sensory neurons, attenuates bombesin-induced suppression of feeding (Ladenheim & Ritter, 1991).

One way bombesin may produce reductions in feeding is by modulating sympathetic activity. Many peptides which modulate food intake do so in a reciprocal relationship to their effects on sympathetic nervous system activity (Bray, 1993). Bray has suggested that peptides that attenuate feeding may do so by increasing sympathetic activity (Bray, 1992). The exact mechanism by which
bombesin may modulate sympathetic activity is unclear. Adrenalectomy abolishes obesity in a variety of experimental paradigms (Tokunaga et al., 1989; Holt & York, 1989), and glucocorticoids have been shown to be necessary components of overeating and obesity (Brown & Fisher, 1986). It appears that adrenalectomy increases sympathetic activity by removing negative feedback modulation of corticotrophin-releasing hormone, thus reversing over consumption and obesity (Vander Tuig, Ohshima, Yoshida, Romsos, & Bray, 1984). While adrenalectomy attenuates some of the behavioral effects of bombesin (Hawkins & Avery, 1983), and peripheral bombesin stimulates adrenal function (Brown, Tache, & Fisher, 1979; Okuma, Yokotani, & Osumi, 1987), the adrenal glands are not necessary for bombesin-induced suppression of feeding (Gibbs, Kulkosky, & Smith, 1981), indicating an extra-adrenal site for possible peripheral bombesin modulation of sympathetic activity.

Effect of Bombesin on Drinking

Central bombesin administration has been demonstrated to suppress drinking in rats at doses that are insufficient to attenuate feeding (Kulkosky, Gibbs, & Smith, 1982). In fact, the majority of reports of investigation of intraventricular bombesin administration on drinking reveals that, with the exception of one
(Ladenheim & Ritter, 1988), all tested paradigms have consistently demonstrated bombesin-induced reductions of water consumption (Babcock, Avery, & Maitland, 1985; de Caro, Massi, Micossi, & Perfumi, 1984; Gibbs, Kulkosky, & Smith, 1981; Kulkosky, Gibbs, & Smith, 1982; Miceli & Malsbury, 1985). Additional evidence has shown that site specific microinjections of bombesin into the lateral hypothalamus, nucleus caudatus putamen, and olfactory tubercle suppress drinking (Willis, Hansky, & Smith, 1984), while others have demonstrated findings contrary to these (Stuckey & Gibbs, 1982). Clearly the preponderance of evidence implicates central administration of bombesin in reduction of drinking behavior. As reported in examining the central effects of bombesin on feeding, the primary explanation for this reduction in drinking is attributed to marked increases in grooming and other competitive behaviors (Miceli & Malsbury, 1985). The exact mechanism by which central bombesin exerts this suppression of drinking remains unknown, but may be related to potential aversive effects.

Findings with peripheral administration of bombesin stand in contrast to those under central administration. In experiments designed to examine the effects of bombesin on feeding and drinking behavior, peripheral administration of bombesin at doses which were demonstrated to suppress feeding failed to alter drinking
Effect of Bombesin on Grooming and Locomotion

Rats given bombesin centrally typically display a set of stereotypies which include: forepaw tremors, "wet-dog" shakes, and excessive grooming (Brown, Rivier, & Vale, 1977). The excessive grooming behavior is characterized by scratching of the head and neck with both forepaw and hindpaw, as well as bathing with saliva. Kulkosky has suggested that bombesin may act as an afferent somatosensory neurotransmitter, and that grooming produced by central administration of bombesin may reflect a response to alteration of cutaneous sensation (Kulkosky, 1988). This increased grooming following central bombesin is also associated with the suppression of feeding seen under these conditions (Kulkosky, Gibbs, & Smith, 1982). In addition, the pattern of behaviors observed after central bombesin do not completely duplicate the behaviors that follow normal satiety, resting decreases while grooming increases (Kulkosky, Gibbs, & Smith, 1982). These changes in behavior are not observed after peripheral bombesin (Kulkosky, Gibbs, & Smith, 1982).
The stereotypies produced by central administration of bombesin appear to be mediated in part by dopaminergic neurons. Increased grooming behavior caused by central bombesin can be attenuated by peripheral administration of fluphenazine and haloperidol (Moody, Merali, & Crawley, 1988). Additional support for this theory is found in data which demonstrates that bombesin-induced grooming abnormalities are abolished by depletion of dopaminergic neurons using 6-dihydroxytryptamine (Moody, Merali, & Crawley, 1988). In addition to a possible dopaminergic axis of bombesin-induced grooming, it is also known that the anxiolytics, diazepam and chlordiazepoxide reverse it as well, implicating a potential involvement of benzodiazepine receptors (Crawley & Moody, 1983; Moody, Merali, & Crawley, 1988).

It has been suggested that the excessive grooming associated with central bombesin administration may represent a behavioral attempt to reduce stress associated with activation of the sympathetic nervous system (Crawley & Moody, 1983). One of the behavioral effects of central bombesin administration is increased locomotion (Pert, Moody, Pert, Dewald, & Rivier, 1980). As previously described in bombesin-induced grooming, it has been found that aberrations in bombesin-induced locomotion can be abolished with dopamine antagonists (Pert, Moody, Pert, Dewald, & Rivier, 1980). While the exact mechanism by
which bombesin produces these behavioral changes is not known, there appears to be an implication of dopaminergic neurons and/or pathways. Interestingly, examination of bombesin-induced hypothermia and hypophagia after microinjection into the substantia nigra, an area critical in dopamine control of motor function, failed to mention any abnormalities in grooming or locomotion (Calisher & Avery, 1984).
BOMBESIN AND THERMOREGULATION

Introduction to Nonshivering Thermogenesis

All mammals are homeothermic, and must maintain their internal body temperature within a narrow range so as to promote optimal homeostasis. There are a variety of factors which impact the regulation of body temperature in homeotherms. Because of this, a brief review of thermogenic systems and thermoregulatory balance is in order.

Several mechanisms provide synergistic effects in the regulation of temperature in homeotherms. One primary thermoregulatory effector is metabolic thermogenesis. Gordon (Gordon, 1990), describes metabolic thermogenesis as being comprised of two primary parts. One component is shivering thermogenesis, in which generation of heat is accomplished by erratic skeletal muscle movements which are not under voluntary control (Gordon, 1990). This form of thermogenesis occurs when an animal is placed in an environment which has an ambient temperature below which normal resting metabolism will not produce sufficient heat to compensate for heat loss (Gordon, 1990).

A second component of metabolic thermogenesis is nonshivering thermogenesis, which is accomplished by activation of special brown adipose tissues commonly found in the interscapular area in rats (Himms-Hagen, 1984), as well as cervical, pericardial, intercostal, and perirenal
areas (Gordon, 1990). Brown adipose tissue is under control of the sympathetic nervous system, and is stimulated to produce heat by both direct innervation and circulating catecholamines (Landsberg, Saville, & Young, 1984). Norepinephrine released from the sympathetic nerves which innervate the brown adipose tissue is the primary initiator of heat production in response to exposure to temperatures below thermoneutrality (Gordon, 1990). Nicholls (Nicholls & Locke, 1984), suggests that in cold exposure the following events are believed to be responsible for acute thermogenesis in brown adipose tissue: 1) norepinephrine binds to the beta receptors, 2) cyclic adenosine monophosphate levels in the cytosol rise, 3) a protein kinase is activated, 4) lipase is phosphorylated and activated, 5) fatty acids are liberated and activated to acylcarnitine, 6) respiratory control inhibits the oxidation of acylcarnitine, and the acylcarnitine and acetyl coenzyme-A pools fill, 7) free fatty acids accumulate, reversing the nucleotide inhibition of the 32,000-Mr (uncoupling) protein, 8) producing an increased proton conductance which allows acylcarnitine to be oxidized, and produces a steady-state concentration of fatty acids with a balance between lipolysis and oxidation. Another thermoregulatory effector is peripheral vasomotor tone. When blood vessels dilate in response to cutaneous cooling, a correlated
increase in metabolism occurs (Gordon, 1990). This vasodilation provides increased circulation to the cutaneous tissues resulting in warming of the periphery. Vasodilation in ambient temperatures below thermoneutrality results in heat dissipation (Gordon, 1987). At temperatures above thermoneutrality, increased vasodilation can result in transfer of environmental energy into the animal, resulting in hyperthermia (Bruck & Zeisberger, 1987).

In addition, evaporative water loss from an organism represents another thermoregulatory effector (Gordon, 1990). As water evaporates, surface cooling takes place. This type of evaporative loss may be passive, such as in the evaporation which takes place under normal respiration, or it may be active, as in the case when rodents spread saliva on their fur to enhance evaporative cooling (Gordon, 1990).

The final and perhaps most common of all thermoregulatory effectors is behavioral actions in which an animal protects its critical body temperature (Gordon, Lee, Chen, Killough, & Ali, 1991). Methods which allow animals to behaviorally influence their body temperature is the use of gradient chambers (Gordon, Lee, Chen, Killough, & Ali, 1991) or operant training for heat reinforcement or heat escape (Hawkins & Avery, 1983).
The event of heat loss or gain can be derived from the "heat balance equation" which comes from the First Law of Thermodynamics (Gordon, 1990). This equation describes the net exchange of heat energy between an organism and its environment. When a homeothermic organism is at rest, heat balance can be described as: 

\[ S = M \pm E \pm R \pm C \pm K \]

Where: 
- \( S \) = net rate of heat storage; 
- \( M \) = metabolic heat production; 
- \( E \) = net rate of evaporative heat exchange; 
- \( R \) = net rate of radiant heat exchange; 
- \( C \) = net rate of convective heat transfer; and 
- \( K \) = net rate of conductive heat transfer (Gordon, 1990). If heat production exceeds heat loss then \( S \) is a positive value, and the animals becomes hyperthermic, whereas if heat loss exceeds production \( S \) is a negative value (Gordon, 1990). A condition of thermal equilibrium is reached when heat loss and heat production are equal (Gordon, 1990). Thus, hypothermia and hyperthermia can be explained in component parts. In the heat balance equation only one factor, metabolism, accounts for internal heat production while several other factors may act simultaneously to contribute or detract from heat storage. Under conditions where ambient temperatures are below thermoneutrality, these other factors generally account for heat loss. At temperatures above thermoneutrality these same factors contribute to heat gain.
Bombesin in the Cold-Exposed Rat

Central injections of bombesin produce hypothermia in cold-exposed rats (Babcock, Barton, & Keene, 1989; Brown, 1981; Brown, Rivier, & Vale, 1977; Francesconi & Mager, 1981; Hawkins & Avery, 1983; Lin & Lin, 1986; Pittman, Tache, & Brown, 1980; Rivier & Brown, 1978; Tache, Pittman, & Brown, 1980; Wakabayashi, Tonegawa, & Shibasaki, 1983; Wunder, Hawkins, Avery, & Swan, 1980). Injections of bombesin into the preoptic area of hypothalamus have been shown to be effective in producing hypothermia in cold-exposed rats (Lin & Lin, 1986; Pittman, Tache, & Brown, 1980; Wunder, Hawkins, Avery, & Swan, 1980). This is an area known to contain thermosensitive neurons (Nakayama, 1985), be an important site for regulation of core body temperature (Cantor & Satinoff, 1976; Satinoff & Rutstein, 1970), and play an active role in stimulation of thermogenesis via brown adipose tissue (Amir & De Blasio, 1991). It has been suggested that bombesin-induced hypothermia in cold-exposed rats may represent an inhibition of regulatory heat production (Brown, 1981; Brown, Allen, & Fisher, 1987; Lin & Lin, 1986). There is also evidence that centrally administered bombesin may reduce metabolism in the cold-exposed animal. Central bombesin produces a reduction in oxygen consumption in cold-exposed rats (Brown, 1981; Lin & Lin, 1986; Shido, Noda, & Nagaoka,
1987; Wunder, Hawkins, Avery, & Swan, 1980), prevents increased oxygen consumption associated with cold-exposure (Brown, 1982), and impairs the cold-induced increase of dopamine accumulation in the interscapular brown adipose tissue, but not other tissues, in rats treated with a dopamine β-hydroxylase inhibitor (Brown, Allen, & Fisher, 1987). Additionally, bombesin causes hypothermia without altering heat loss as measured by cutaneous temperatures (Lin & Lin, 1986).

The exact mechanism by which bombesin induces hypothermia at temperatures below thermoneutrality is unclear. Findings have established that thyrotropin releasing factor, prostaglandin E₂, and naloxone reverse bombesin-induced hypothermia in cold-exposed animals (Brown, Rivier, & Vale, 1977). Additionally, bombesin prevents cold-induced secretion of thyrotropin stimulating hormone, while not preventing thyrotropin releasing factor-induced secretion of thyrotropin stimulating factor (Rivier & Brown, 1978). This implicates a pituitary axis of bombesin control of thyrotropin stimulating hormone. Yet, bombesin injected centrally produces hypothermia in cold-exposed rats that have been hypophysectomized (Rasler, 1983). The latter data would indicate that bombesin-induced hypothermia in animals exposed to cold is not dependent on centrally mediated release of pituitary hormones.
Given the previously mentioned information about bombesin-induced hypothermia in animals tested at temperatures below thermoneutrality, it would appear that bombesin likely produces hypothermia by interfering with compensatory heat production. The agents known to reverse bombesin-induced hypothermia under these conditions all contribute to increased metabolism. Knowing that energy balance is a sum of heat production and heat loss, and that bombesin has been repeatedly determined to reduce or impair metabolism as measured by oxygen consumption in cold-exposed rats, it would appear that bombesin most likely produces hypothermia by reducing heat production. Yet, there is evidence to the contrary. Bombesin injected centrally has been found to increase tail skin temperatures in animals at normal ambient temperatures (Lin & Lin, 1986; Tache, Pittman, & Brown, 1980). While these findings are not apparent at temperatures below thermoneutrality, it does not preclude heat loss as a mechanism by which bombesin may induce hypothermia. Tache has suggested that bombesin may act to produce hypothermia in cold-exposed conditions by essentially making the animals poikilothermic, and that change in body temperature is dependent upon ambient temperatures (Tache, Pittman, & Brown, 1980). In support of this are findings that bombesin selectively stimulates warm-responsive neurons in the preoptic anterior hypothalamic area (Lin &
Lin, 1986). This type of activation of warm-responsive neurons would result in vasodilation and other physiological changes to promote heat loss (Boulant & Dean, 1986). Under this type of activity it would seem likely that ambient temperature would then influence core body temperature. Increased blood flow to cutaneous areas would allow for transfer of heat across a gradient. In cold-exposed animals heat would be lost to the environment while animals tested at temperatures above the upper level of thermoneutrality would gain heat. Additionally, animals kept at ambient temperatures approximating normal body temperatures would not experience a transfer of heat energy. This type of thermal exchange in core body temperatures has been found to exist in animals administered bombesin centrally. Tache and colleagues (Tache, Pittman, & Brown, 1980) found that central bombesin produced hypothermia in animals tested at an ambient temperature of 4° C, hyperthermia at an ambient temperature of 36° C, and euthermia at an ambient temperature of 31° C. Additionally, bombesin treated animals which become hyperthermic at temperatures above thermoneutrality can be made hypothermic by transfer to a cold environment (Tache, Pittman, & Brown, 1980).

It has been suggested that bombesin may alter thermoregulatory "set point"; a core body temperature established by central mechanisms which an animal will
attempt to defend (Avery & Calisher, 1982; Wunder, Hawkins, Avery, & Swan, 1980). Animals trained to bar press for heat escape, do so more frequently after central bombesin administration (Avery, Hawkins, & Wunder, 1980). Avery reasons that this response is a behavioral attempt to protect a lowered thermoregulatory set point. Given that bombesin activates warm-responsive neurons, as previously demonstrated (Lin & Lin, 1986), then it would seem plausible that these animals may "feel" warm, and thus bar press to escape heat. In support of this, animals administered bombesin centrally have been found to select ambient temperatures in gradients significantly lower than saline injected animals (Stump, McCoy, & Avery, 1990). Yet, in the case of bar pressing for defense of an altered set point, there is contrary data. It has been found that adrenalectomy abolishes increased bar pressing for heat escape in bombesin treated animals tested at temperatures below thermoneutrality (Hawkins & Avery, 1983). Hawkins reasons that bombesin produces an increase in behavioral activation secondary to stimulation of the adrenal glands. This seems unlikely due to findings that central bombesin results in decreased bar pressing for food reward (Babcock, Avery, & Maitland, 1985). If bombesin simply increases rewarded behavior, then it would follow that this response would be generalized to other paradigms, which is not the case. Furthermore,
adrenalectomy is known to increase thermogenesis in brown adipose tissue (Kim & Romsos, 1990). This may have been the case as none of the animals tested by Hawkins ever actually displayed hypothermia (Hawkins & Avery, 1983). In fact, adrenalectomized rats did not display any differences in heat escape after central bombesin versus control treatment (Hawkins & Avery, 1983), which was quite probably because they were euthermic.

Provided the previous findings related to bombesin-induced hypothermia in animals tested at temperatures below thermoneutrality, the underlying mechanism is still not proven. There is evidence which suggests that central bombesin injections may produce hypothermia by activation of brain mechanisms, thereby disrupting metabolic processes or increasing heat loss via peripheral vasodilation. In addition there is evidence that bombesin may alter central mechanisms in a way such that an animal displays behaviors that appear to be directed toward defense of a lowered core body temperature. The possibility that more than one of these factors may influence bombesin-induced hypothermia in cold environments has not been ruled out.
Bombesin-Induced Hypothermia at Normal Ambient Temperatures

Central injection of bombesin was first reported to produce hypothermia in rats tested at normal ambient temperatures in 1980 (Tache, Pittman, & Brown, 1980). It was later reported that bombesin-induced hypothermia in animals tested near thermoneutrality was associated with no change in metabolic rate as measured by oxygen consumption (Brown, 1981; Wunder, Hawkins, Avery, & Swan, 1980). While some data exists suggesting that hypothermia observed under these conditions is not dependent upon manipulation of food intake (Tache, Pittman, & Brown, 1980; Wunder, Hawkins, Avery, & Swan, 1980), others were unable to replicate these findings (Avery & Calisher, 1982; Babcock & Barton, 1989; Babcock & Barton, 1990; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock, Barton, Perez, & Hearndon, 1989; Barton & Babcock, 1990; Barton & Hawkins, 1993; Calisher & Avery, 1984). To date, bombesin has been reported to produce hypothermia when injected into the cerebral ventricles (Avery & Calisher, 1982; Babcock, Barton, & Keene, 1989; Babcock, Barton, Perez, & Hearndon, 1989; Babcock & Wunder, 1984; Francesconi & Mager, 1981; Wunder, Hawkins, Avery, & Swan, 1980), the preoptic area of the hypothalamus (Babcock, Baker, & Moody, 1992; Babcock & Barton, 1989; Babcock, Barton, Gunion, &
Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock & Maisel, 1991; Barton & Babcock, 1990), the hypothalamic paraventricular nucleus (Babcock & Barton, 1990), and the substantia nigra (Calisher & Avery, 1984), of animals tested at normal ambient temperatures.

The prevailing theory is that bombesin-induced hypothermia in animals tested under normal ambient temperatures is dependent on some pre-existing condition, which acts as a "permissive" factor for hypothermia to occur. Previous data has established that animals acutely food-deprived (Avery & Calisher, 1982; Babcock, Baker, & Moody, 1992; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock & Wunder, 1984; Barton & Babcock, 1990; Calisher & Avery, 1984), injected with insulin peripherally (Babcock & Barton, 1989; Babcock & Barton, 1990; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, Perez, & Hearndon, 1989; Barton & Babcock, 1990), or 2-deoxy-D-glucose (Babcock & Maisel, 1991), or with lesions of the ventromedial hypothalamus (Barton & Hawkins, 1993), experience hypothermia when injected with bombesin centrally. Exactly how these pre-existing conditions contribute to the production of bombesin-induced hypothermia at normal ambient temperatures remains unclear. It has been theorized that one potential condition these manipulations may contribute to is reduced peripheral glucose availability (Babcock &
Maisel, 1991). This appears to be supported by findings that bombesin-induced hypothermia seen in animals acutely deprived of food or treated with peripheral insulin is attenuated by allowing the animals to eat (Barton & Babcock, 1990).

Bombesin-induced hypothermia demonstrated at normal ambient temperatures is believed to be mediated by different mechanisms from those seen in conditions of acute cold exposure. Several findings contribute to this belief. Unlike bombesin-induced hypothermia observed in cold-exposed animals, hypothermia precipitated by bombesin at normal ambient temperatures is not attenuated by peripheral naloxone (Babcock, Barton, & Keene, 1989). Moreover, hypothermia produced by bombesin at normal ambient temperatures is not associated with a decrease in metabolism (Babcock & Wunder, 1984), yet conflicting data exists (Lin & Lin, 1986; Shido, Noda, & Nagasaka, 1987; Tache, Pittman, & Brown, 1980).

The mechanism by which bombesin produces hypothermia at normal ambient temperatures is unclear. Lin (Lin & Lin, 1986), has demonstrated that bombesin-induced hypothermia under conditions of normal ambient temperature results in reduced oxygen consumption and increased heat loss. In opposition to Lin's data, experiments examining bombesin-induced heat loss related to hypothermia using cutaneous tail temperature failed to support this finding.
(Babcock & Barton, 1989). If bombesin does not alter metabolic rate, then some form of heat loss must account for the hypothermia produced. In support of this theory are findings that the core body temperature of rats injected centrally with bombesin drift toward the temperature of their environment (Tache, Pittman, & Brown, 1980). Additional support is found in data which establishes that bombesin produces peripheral vasodilation (Lin & Lin, 1986). It has also been shown that bombesin produces increased evaporative heat loss as measured by experiments designed to isolate this parameter via direct calorimetry (Shido, Noda, & Nagasaka, 1987).

Thus, given the previous findings, it is clear that bombesin can produce hypothermia at normal ambient temperatures. Bombesin-induced hypothermia under these conditions appears to be reliant upon some manipulation of factors related to nutritional status, which have been suggested to negatively influence glucose availability. Reports that bombesin alters resting metabolic rate out number ones that it does not. In addition, there is considerable evidence that bombesin promotes heat loss at normal ambient temperatures.

The relationship between the permissive events which contribute to bombesin-induced hypothermia at normal ambient temperatures is shown in Figure 2. Acute food-deprivation or peripheral injection of
2-deoxy-D-glucose reduce glucose utilization while having little affect on glucose availability. Both peripheral injection with insulin or lesions of the ventromedial hypothalamus increase utilization but with differential affect on availability. Insulin injection reduces availability by promoting utilization while lesions of the ventromedial hypothalamus increases availability as well as utilization. Thus, there is no consistent relationship between the necessary pre-existing events which allow bombesin-induced hypothermia at normal ambient temperatures and their affect on glucose utilization or availability.
Figure 2: Relation of permissive events to glucose utilization and availability.

**Glucose Availability**
- Little to no net effect.
- Reduces availability.
- Little to no net effect.
- Increases availability.

**Glucose Utilization**

1. Acute Food-Deprivation
   - Reduces utilization.

2. Insulin-Injection
   - Increases utilization.

3. 2DG-Injection
   - Reduces utilization.

4. Lesions of the VMH
   - Increases utilization.
PROBLEM STATEMENT

Bombesin is known to produce a wide spectrum of behaviorally and physiologically related effects when administered centrally. Previous studies of bombesin-induced hypothermia have been conducted under conditions of cold-exposure, food-deprivation, insulin treatment, 2-deoxy-D-glucose treatment, and lesions of the ventromedial hypothalamus. Each of these experimental manipulations result in hypothermia following central bombesin administration, yet the underlying mechanisms are not well understood. The objective of this study was to determine if a potential reduction of sympathetic nervous system stimulation, brought about by the factors previously shown to be permissive events, is responsible for bombesin-induced hypothermia at normal ambient temperatures.

All of the conditions which have been implicated as permissive factors in bombesin-induced hypothermia produce reductions in efferent sympathetic nervous system activation of interscapular brown adipose tissue. Food-deprivation (Dulloo, Young, & Landsberg, 1988; Landsberg & Young, 1978; Sakaguchi, Arase, Fisler, & Bray, 1988) and injections of 2-deoxy-D-glucose (Egawa, Yoshimatsu, & Bray, 1989; Holt & York, 1989; Landsberg & Krieger, 1989) result in decreased norepinephrine turnover in brown adipose tissue and reduce firing rates
of isolated efferent sympathetic nerves that innervate brown adipose tissue, as do lesions of the ventromedial hypothalamus (Niijima, Rohner-Jeanrenaud, & Jeanrenaud, 1984). Additionally, hyperinsulinemic animals also exhibit reductions in norepinephrine turnover in brown adipose tissue (Nishioka, Yoshida, Yoshioka, & Kondo, 1987). Thus, conditions that permit bombesin-induced hypothermia in animals tested at normal ambient temperatures, may do so by attenuating the thermogenic capacity of brown adipose tissue to compensate for peripheral heat loss. This hypothesis receives indirect support by findings that bombesin-induced hypothermia is attenuated when animals are allowed to eat (Barton & Babcock, 1990). Feeding promotes sympathetic nervous system activation of brown adipose tissue (Sakaguchi, Arase, Fisler, & Bray, 1988) and increased heat production (Himms-Hagen, Hogan, & Zaror-Behrens, 1986; Rothwell & Stock, 1979; Stock & Rothwell, 1986), as well as its growth (Sundin & Nechad, 1983). Therefore, it seems plausible that attenuation of bombesin-induced hypothermia in food-deprived or insulin-treated rats allowed to feed during testing is mediated by increased thermogenesis in the brown adipose tissue, secondary to food consumption.

If reduced sympathetic nervous system stimulation of brown adipose tissue is necessary for bombesin-induced hypothermia, then ganglionic blockade of the sympathetic
efferents which innervate brown adipose tissue in rats administered bombesin, should result in hypothermia at normal ambient temperatures. Chlorisondamine administered peripherally acts on nicotinic receptors within the sympathetic ganglia to block sympathetic nervous system activity and prevent increased thermogenesis (Amir, 1990,a; Amir, 1990,b; Amir, 1990,c; Amir & De Blasio, 1991). In addition, it does not modulate serum glucose levels or glucose utilization (Turinsky, 1974). Therefore, ad-libitum fed rats were injected centrally with bombesin and peripherally with chlorisondamine to investigate the effect of bombesin on core body temperature.

If reductions in sympathetic nervous system stimulation to brown adipose tissue allow bombesin to produce hypothermia at normal ambient temperatures, then stimulation of the brown adipose tissue by way of a sympathomimetic, should block bombesin-induced hypothermia. The experimental compound CL-316,243 is a drug that acts on \( \beta_3 \)-adrenergic receptors and has been demonstrated to promote thermogenesis in brown adipose tissue (Himms-Hagen et al., 1994). Chronic treatment of rats on high fat diets with this compound produces an increase in metabolic rate, hypertrophy of brown adipose tissue, and a robust increase in uncoupling protein (Himms-Hagen et al., 1994). Himms-Hagen (Himms-Hagen et
al., 1994), has theorized that CL-316,243 induces an increase in total mitochondria in chronic application, thereby producing the increase in uncoupling protein. Uncoupling protein promotes oxidation of substrate which is no longer coupled to adenosine triphosphate synthesis, thereby providing a unique source of dissipative heat generation in brown adipose tissues (Klingenberg, 1990). Thus, peripherally administered CL-316,243 was used to block the potential effects of reduced sympathetic nervous system activation associated with bombesin-induced hypothermia at normal ambient temperatures.

**Hypotheses**

The following hypotheses will be addressed under the proposed experiments:

**Hypothesis 1.** If reductions in sympathetic nervous system activation of brown adipose tissue provide a necessary pre-existing condition for bombesin-induced hypothermia in animals tested at normal ambient temperatures, then ad-libitum fed rats, administered chlorisondamine peripherally, should become hypothermic after lateral ventricular injections of bombesin.

**Hypothesis 2.** If reduction in sympathetic nervous system activation of brown adipose tissue thermogenesis acts as the permissive event for bombesin-induced hypothermia at normal ambient temperatures, then CL-316,243 administered
peripherally should prevent bombesin-induced hypothermia in food-deprived and chlorisondamine treated animals injected with bombesin into the lateral ventricles.
GENERAL METHODS

Subjects

In all experiments male Sprague-Dawley rats (300-400 g) were used. Animals were housed individually in standard wire and steel laboratory cages. Animals received ad-libitum access to standard laboratory chow (Ralston Purina Co.) and water except where mentioned in specific experimental designs. Ambient temperatures for housing and testing procedures was maintained at 24° C (± 2° C). Photoperiod was on a twelve hour schedule with onset of the dark-phase at 19:00 hrs.

Surgery

A guide cannulae constructed of 22-gauge stainless-steel tubing was implanted into a lateral cerebral ventricle of each animal under pentobarbital anesthesia (35 mg/Kg body weight, i.p.). After initiation of anesthesia animals were placed in a stereotaxic instrument (model 900A, David Kopf Instruments, Tujunga, CA) and a midline incision made on the superior surface of the skull. The skull was trephined and stainless-steel anchor screws inserted into the bone. Guide cannulae were implanted using stereotaxic coordinates 0.9 mm posterior to bregma, 1.5 mm lateral to midline, and 4.0 mm ventral to the skull surface (Paxinos & Watson, 1982). Cannulae were cemented in place using dental acrylic. A 26-guage
wire obturator was kept in the guide cannula to maintain patency except during injections. All animals were provided a 7-10 day post-surgical recovery period prior to experimentation.

Adrenalectomy was performed under pentobarbital anesthesia (35 mg/Kg body weight, i.p.). After initiation of anesthesia, the surgical area was prepared and incisions parallel to the spine, caudal to the back muscles, and immediately posterior to the rib cage were made. In the event of complete bilateral adrenalectomy, exploration and isolation of the adrenals was accomplished using curved forceps. The adrenal glands were removed by blunt dissection and the muscle and skin closed using interrupted sutures. In sham adrenalectomized subjects, the adrenals were visualized and the incisions were sutured closed. Following surgery all animals were allowed a 7-10 day recovery period and were maintained on sterile 0.9% saline drinking solution in place of tap water.

Drugs

Anhydrous bombesin (Research Biologicals Inc., CA) was diluted with 0.9% sterile saline (100 ng/5.0 µl) for icv administration. Injections were be made using a 26-guage injector cannula designed to extend 1.0 mm beyond the ventral aspect of the guide cannula. The injector
cannula was connected to a 100 μl syringe by sterile polyethylene tubing. Injection volumes (5.0 μl) were infused over a 1 minute period of time using a motor driven infusion pump (Harvard Apparatus, South Natick, MA).

Anhydrous chlorisondamine HCl (CIBA Pharmaceuticals, Summit, NJ) were diluted with 0.9% sterile saline (2.5 mg/1 ml) for i.p. injection. Anhydrous CL-316,243 (American Cyanamid, Wayne, NJ) was diluted with 0.9% sterile saline (1.0 mg/1 ml) for i.p. injection.

Crystalline corticosterone (ICN Biomedicals, Costa Mesa, CA) was dissolved in polyoxyethylenesorbitan monolaurate (Tween 20) (Sigma, St. Louis, MO) and then diluted with sterile saline in a 1:5 ratio (0.1 mg/ml). Peripheral injections were made using a 1 ml syringe and 26-guage hypodermic needle.

**Cannulae Verification**

At the completion of testing, animals were sacrificed by carbon dioxide euthanasia. An injector was placed into the guide cannula and a 5.0 μl volume of India ink infused. Brains were removed from craniums and coronal sections made to examine the ventricles for ink infusion. Only data coming from those animals having ink infusion into the lateral cerebral ventricles were used for analysis.
Statistics

Data was evaluated using analysis of variance (ANOVA) using the computer program SigmaStat (Jandel Scientific, San Rafael, Ca). Differences between group means were analyzed using Newman-Keuls post hoc test.
EXPERIMENT I

Introduction

Bombesin-induced hypothermia in animals tested at normal ambient temperatures appears to require an antecedent event(s) which has been linked to reduction in sympathetic stimulation of brown adipose tissue. The importance of brown adipose activity in the induction of bombesin-induced hypothermia is unknown. This study evaluated core body temperature using peripheral chlorisondamine injections to block ganglionic innervation of the brown adipose tissue prior to central bombesin administration in ad-libitum fed rats.

Procedure

Beginning at 09:30 animals were removed from their cages and core body temperatures were measured by inserting a thermistor probe (Harvard Apparatus, South Natick, MA) 6.5 cm beyond the anal orifice. Immediately after recording temperatures each animal was injected with either chlorisondamine (2.5 mg/kg) or saline, and returned to its cage. Thirty minutes after peripheral injection the animals were again removed from their cages, core body temperatures recorded, received central injection of either bombesin (100 ng/5.0 µl, icv) or saline vehicle (5.0 µl), and then returned to their cages. After 60 minutes had elapsed from central injection, core body
temperature was recorded to evaluate potential hypothermia. A between-group design was utilized resulting in four groups: (central/peripheral) saline/saline, bombesin/saline, bombesin/chlorisondamine, and saline/chlorisondamine (n=7 per group).

Results

Change in core body temperature defined as the difference from preinjection levels are shown in Figure 3. Two-way repeated measures ANOVA demonstrated a significant effect of treatment condition on core body temperature \( F(3,48)=12.897, p<0.001 \), as well as a significant effect of time of recording on core body temperature \( F(2,48)=24.851, p<0.001 \). Additionally, there was a significant interaction between treatment condition and time \( F(6,48)=20.033, p<0.001 \). Newman-Keuls post hoc testing revealed that body temperature did not differ significantly between groups as a function of peripheral injection alone, and that only animals receiving the combination of central bombesin and peripheral chlorisondamine became hypothermic.
Figure 3. Change in core temperature in ad libitum fed rats treated with bombesin and chlorisondamine. BEFORE, represents change in core temperature at time of central injection (30 min post peripheral injection). AFTER, represents change in core temperature at termination of experiment (60 min post central injection). Legend indicates central/peripheral treatment [bombesin (BOM) 100 ng/5μl, icv; chorisondamine (CHL) 2.5 mg/kg, ip; vehicle (SAL)]. Bars indicate standard error of the mean. *p<0.05 vs SAL/SAL control condition.
EXPERIMENT II

Introduction

Experiment I indicates that bombesin-induced hypothermia occurs in ad-libitum fed animals treated with the nicotinic ganglionic receptor blocker, chlorisondamine. It is hypothesized that this hypothermia is secondary to attenuation of sympathetic drive to brown adipose tissue. This study examines the hypothesis that direct activation of thermogenesis in brown adipose tissue, in animals which have received chlorisondamine, can attenuate bombesin-induced hypothermia. This was accomplished using peripheral injections of the $\beta_3$-adrenergic agonist, CL-316,243.

Procedure

Beginning at 09:30 animals were removed from their cages and core body temperatures were measured by inserting a thermistor probe 6.5 cm beyond the anal orifice. Immediately after recording temperatures each animal was injected with either chlorisondamine (2.5 mg/kg) and saline, or chlorisondamine (2.5 mg/kg, ip) and CL-316,243 (1 mg/kg, ip) and returned to its cage. Thirty minutes after peripheral injections the animals were removed from their cages, core body temperatures recorded, were administered central injection of either bombesin (100 ng/5.0 µl, icv) or saline vehicle (5.0 µl), and then
returned to their cages. After 60 minutes had elapsed from central injection, the core body temperature was recorded to evaluate potential hypothermia. A between-group design was utilized resulting in four groups: (central/peripheral) saline/chlorisondamine+saline, saline/chlorisondamine+CL-316,243, bombesin/chlorisondamine+saline, or bombesin/chlorisondamine+CL-316,243, (n=7 per group).

Results

Change in core body temperature after treatment with bombesin and CL-316,243 is shown in Figure 4. Two-way repeated measures ANOVA indicated a significant effect of treatment condition on core body temperature \( [F(3,72)=4.05, p<0.01] \), time of temperature recording on core body temperature \( [F(2,72)=8.26, p<0.001] \), and interaction between treatment condition and time of temperature recording \( [F(6,72)=3.93, p<0.001] \). Newman-Keuls post hoc testing indicates that core body temperature did not differ between groups as a function of peripheral injection alone. The only group that demonstrated significant hypothermia was the one that received chlorisondamine + saline peripherally, and bombesin centrally. Peripherial injection of CL-316,243 prevented hypothermia in animals receiving central bombesin following peripheral chlorisondamine treatment.
Figure 4. Change in core temperature in ad libitum fed rats following central bombesin and peripheral chlorisondamine and CL-316,243. BEFORE, represents change in core temperature at time of central injection (30 min post peripheral injection). AFTER, represents change in core temperature at termination of experiment (60 min post central injection). Inset legend indicates central/peripheral treatment [bombesin (BOM) 100 ng/5μl, icv; chlorisondamine (CHL) 2.5 mg/kg, ip; CL-316,243 (CL)1.0 mg/kg; ip; vehicle (SAL)]. Bars indicate standard error of the mean for each condition and time point. *p < 0.05 vs SAL/CHL+SAL control condition.
EXPERIMENT III

Introduction

Previous studies have demonstrated that bombesin-induced hypothermia occurs in acutely food-deprived animals tested at normal ambient temperatures (Avery & Calisher, 1982; Babcock, Baker, & Moody, 1992; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock & Wunder, 1984; Barton & Babcock, 1990; Calisher & Avery, 1984). Acute food deprivation is known to result in reduced sympathetic nervous system drive to brown adipose tissue (Sakaguchi, Arase, Fisler, & Bray, 1988) but not produce hypoglycemia (Barton & Babcock, 1990). Experiment II demonstrated that the $\beta_3$-adrenergic sympathomimetic CL-316,243 prevents bombesin-induced hypothermia in ad-libitum fed rats treated with chlorisondamine, most likely by direct stimulation of thermogenesis in brown adipose tissue. This experiment will examine if food-deprivation produces a permissive event in bombesin-induced hypothermia by reducing sympathetic stimulation of brown adipose tissue and if alteration of serum glucose is important in this event. If suppression of sympathetic nervous system activity is the pivotal mechanism in bombesin-induced hypothermia, then peripheral injections of CL-316,243 should prevent hypothermia under these conditions.
Procedure

Animals were food-deprived 20 hours prior to central injections except for a single control group (n=5). Techniques utilized for recording core body temperature and animals handling are identical to those detailed in Experiment I. Core body temperature was recorded at 30 minutes prior to central injection (beginning at 0930), at the time of central injection, and 30 and 60 minutes post central injection. Peripheral injections consisted of either saline, or CL-316,243 (1 mg/Kg, i.p.). Central injections consisted of either bombesin (100 ng/5.0 ul, icv) or saline (5.0 ul). A between-group design was utilized resulting in the following drug treatment groups at specific times: (condition time relative to central injection) ad libitum fed, food-deprived, food-deprived+i.p. saline, food-deprived+i.p. CL-316,243, icv saline+i.p. saline, icv saline+i.p. CL-316,243, icv bombesin+i.p. saline, icv bombesin+i.p. CL-316,243, icv saline+i.p. saline, icv saline+i.p. CL-316,243, icv bombesin+i.p. saline, icv bombesin+i.p. CL-316,243. This resulted in 12 groups of animals (n=5 per group). This design can be seen in Table 1.

Blood samples were collected by decapitation of live animals and draining trunk blood into sterile test tubes in an ice chilled container. The blood was centrifuged at 3500 rpm for 5 minutes using a TRIAC centrifuge, model
Matrix of test conditions. Rows represent treatment conditions (central/peripheral). Lower 4 rows represent food-deprivation (20 hr) prior to testing. Columns represent time of data collection relative to central injection. Group data from subjects prior to central or peripheral injection (CON) depicted in far left column. Each cell represents an experimental group (n = 5/group). Groups experienced temperature data collection for all time points to the left of, and including the column of listing.
number 0200 (Clay Adams, Parsippany, NJ). The supernatant was collected and stored at -70° C until assay. Glucose assays were performed using the Beckman CX5 UV-hexokinase method in the Clinical Research Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA.

Results

Change in core body temperature secondary to treatment condition and time are shown in Table 2. Analysis of variance indicate that there is a significant effect of treatment condition on core body temperature [F(11,48)=21.8, p< 0.0001]. Newman-Keuls post hoc testing demonstrated that only the group which received bombesin centrally and saline peripherally became hypothermic, that they were more hypothermic at 60 minutes after central injection than at 30 minutes, and that peripheral injection with CL-316,243 prevented bombesin-induced hypothermia at both time points. Additionally, a two-way repeated measures ANOVA performed on the four groups which progressed through all temperature measurement points demonstrated a significant effect of treatment condition on core body temperature [F(3,48)=18.3, p<0.0001], as well as on time of recording [F(3,48)=34.3, p<0.0001], and interaction between treatment condition and time or recording [F(9,48)=20.8, p<0.0001]. The change in core temperature in these groups can be seen in Figure 5.
Table 2: Core Body Temperature for Treatment Groups Relative to Injection Time and Condition.

<table>
<thead>
<tr>
<th>Condition</th>
<th>-30 min</th>
<th>0 min</th>
<th>+30 min</th>
<th>+60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>38.2±0.139</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD CON</td>
<td>38.4±0.121</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD -/SAL</td>
<td>38.2±0.164</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD -/CL</td>
<td>38.0±0.226</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD SAL/SAL</td>
<td>37.8±0.27</td>
<td>37.9±0.213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD SAL/CL</td>
<td>38.1±0.178</td>
<td>38.0±0.124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD BOM/CL</td>
<td>37.8±0.279</td>
<td>38.1±0.233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD BOM/SAL</td>
<td>*36.1±0.275</td>
<td>**34.5±0.499</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lower 7 rows (FD) represents food-deprivation (20 hr) prior to testing. Treatment conditions (central/peripheral): SAL = saline, BOM = bombesin, CL = CL-316,243, CON = groups which received no injections. Each cell represents an experimental group (n = 5/group) (°C+SEM). *p<0.05 from all other conditions. **p<0.05 from BOM/SAL condition at 30 min.
Data from glucose analysis can be seen in Table 3. One-way ANOVA on glucose data revealed that there was a significant effect of sample condition on glucose levels \[ F(11,48)=13.798, \ p<0.001 \]. Newman-Keuls post hoc testing revealed that groups receiving saline centrally and CL-316,243 peripherally had significantly lower serum glucose levels than all other groups. Rats which were acutely deprived of food had glucose levels no different from animals allowed ad libitum access to food. Additionally, central injection of bombesin prevented the reduction of serum glucose levels seen in animals treated with CL-316,243 peripherally.
Figure 5. Change in core temperature in acutely fasted rats following central bombesin and peripheral CL-316,243. Abscissa categories indicate change in temperature collection relative to time of central injection (0, 30, 60 min). Inset legend indicates central/peripheral treatment [bombesin (BOM) 100 ng/5 μl, icv; CL-316,243 (CL) 1 mg/kg, ip; vehicle (SAL)]. Bars indicate standard error of the mean for each condition and time point.

*p< 0.05 vs SAL/SAL control condition.

**p< 0.05 vs BOM/SAL condition at 30 min.
Table 3: Mean Serum Glucose Levels for Treatment Groups Relative to Injection Time and Condition.

<table>
<thead>
<tr>
<th>Condition</th>
<th>-30 min</th>
<th>0 min</th>
<th>+30 min</th>
<th>+60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ad-lib fed</td>
<td>142.2±3.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food-deprived</td>
<td>151.0±2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD SAL/CL</td>
<td></td>
<td>*60.8±1.94</td>
<td>*72.6±5.1</td>
<td>*79.0±2.39</td>
</tr>
<tr>
<td>FD SAL/SAL</td>
<td>130±3.99</td>
<td>121.2±2.96</td>
<td>112.4±1.33</td>
<td></td>
</tr>
<tr>
<td>FD BOM/SAL</td>
<td>141.4±10.5</td>
<td>150.2±16.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD BOM/CL</td>
<td>122.0±13.62</td>
<td>109.6±12.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Serum glucose levels (mg/DL ± SEM) for treatment conditions at time points relative to central injection (columns). Treatment conditions (central/peripheral): SAL = saline, BOM = bombesin, CL = CL-316,243. Prefix FD represent data from rats food-deprived (20 hr) prior to treatment conditions. *p<0.05 from ad-lib fed controls.
EXPERIMENT IV

Introduction

The sympathetic nervous system can modulate serum glucose levels by neural innervation of the adrenal medulla (Clutter, Rizza, Gerich, & Cryer, 1988). Hypothalamic stimulation of the sympathetic nervous system causes the production and release of epinephrine, which in turn causes release of glucose from the liver (Guyton, 1991). Stress is known to increase the release of corticosterone in rats (Mormede et al., 1990) and elevations in corticosterone levels are known to suppress sympathetic nervous system activation of brown adipose tissue (Davidovic, Vasilev, & Stojanovic-Susulic, 1992). In addition, central bombesin administration produces hyperglycemia secondary to stimulation of release of epinephrine from the adrenal medulla (Brown, Tache, & Fisher, 1979). Previous experiments have demonstrated that adrenalectomized animals become hypothermic after central bombesin under conditions of food-deprivation (Barton, unpublished) but, confirmation of adrenalectomy was not performed. Thus, the contribution of adrenal products, specifically epinephrine, on bombesin-induced hypothermia at normal ambient temperatures is unknown. This study will examine the impact of bombesin in adrenalectomized rats.
Procedure

Animals were food-deprived 20 hours prior to central injections. Adrenalectomized animals received corticosterone replacement (0.1 mg/kg, s.q.) (Tempel, McEwen, & Leibowitz, 1992) while sham adrenalectomized animals received vehicle injections (1.0 ml/kg, s.q.) at 0500 each day after peripheral surgery. All animals were maintained on saline drinking solution. Techniques utilized for recording core body temperature and animals handling are identical to those detailed in Experiment I. Core body temperature was recorded at the time of central injection and 60 minutes post central injection. This resulted in a between-subjects design with three experimental groups tested under the following conditions: (surgical treatment+central injection/peripheral injection) sham adrenalectomy+vehicle/vehicle, sham adrenalectomy+bombesin/vehicle, and adrenalectomy+bombesin/corticosterone (n=7 per group).

Immediately following the last data collection period animals were sacrificed and trunk blood collected in ice chilled test tubes. Serum was collected and stored as described in Experiment III. Confirmation of complete adrenalectomy was established by serum aldosterone assay. Aldosterone assays were performed using Aldosterone-MAIA in vitro radioimmunoassay diagnostic kits (CIBA-Corning Diagnostics Corp., East Walpole, MA), in the Clinical
The criterion for complete adrenalectomy was serum aldosterone levels below 5 pg/ml.

**Results**

Figure 6 depicts changes in core body temperature after bombesin treatment in food-deprived rats with and without adrenalectomy. Analysis of data using two-way, repeated measures ANOVA demonstrated a significant effect for treatment condition \([F(2,18)=26.9, p<0.0001]\), time of recording \([F(1,18)=161.9, p<0.0001]\), and interaction between the two \([F(2,18)=88.8, p<0.0001]\). Newman-Keuls post hoc testing revealed that food-deprived animals became hypothermic 60 minutes after central bombesin injection with or without adrenalectomy, and that adrenalectomized animals were significantly more hypothermic than sham adrenalectomized animals after bombesin treatment.
Figure 6. Change in core body temperature in acutely fasted rats following central bombesin with and without adrenalectomy. Inset legend indicates peripheral surgical treatment + central injection/peripheral injection [sham adrenalectomy (SADX); adrenalectomy (ADX); bombesin (BOM) 100 ng/5μl, icv; corticosterone (CORT) 0.1 mg/kg, sc; vehicle (VEH)]. Bars indicate standard error of the mean. * p< 0.05 vs SADX+VEH/VEH control condition. ** p< 0.05 vs SADX+BOM/VEH condition.
DISCUSSION

The four experiments conducted in this dissertation provide robust evidence that bombesin produces hypothermia in rats tested at normal ambient temperatures under conditions in which the normal reflex sympathetic activation of brown adipose tissue thermogenesis is blunted. In Experiment I, it was proven that bombesin-induced hypothermia could be produced under conditions of ad-libitum access to food by using the ganglionic blocker chlorisondamine. Chlorisondamine is known to act peripherally to prevent activation of brown adipose tissue thermogenesis by suppressing sympathetic drive (Amir, 1990,a; Amir, 1990,b; Amir, 1990,c; Amir & De Blasio, 1991). Additionally, chlorisondamine does not effect serum glucose levels or glucose utilization (Turinsky, 1974). Data from Experiment II demonstrates that bombesin produces hypothermia when administered centrally to ad-libitum fed rats treated peripherally with chlorisondamine, as in Experiment I, and that this hypothermia is prevented by addition of peripheral treatment with the β1-receptor agonist, CL-316,243. The sympathomimetic, CL-316,243 is known to act selectively on β3-adrenergic receptors (Bloom et al., 1992) which are found primarily in adipose tissues, and produce thermogenic responses when activated (Himms-Hagen et al., 1994). In Experiment III, central bombesin injection
produced hypothermia in acutely food-deprived rats tested at normal ambient temperatures, confirming previous reports (Avery & Calisher, 1982; Babcock, Baker, & Moody, 1992; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock & Wunder, 1984; Barton & Babcock, 1990; Calisher & Avery, 1984), and this hypothermia was prevented by peripheral treatment with CL-316,243. In addition, serum glucose data from Experiment III establishes that peripheral treatment with CL-316,243 produces hypoglycemia relative to food-deprived animals receiving saline control treatment or ad-libitum fed rats, and that central bombesin attenuates this hypoglycemia. Experiment IV demonstrated that bombesin produces hypothermia in acutely food-deprived adrenalectomized rats with corticosterone replacement, and in sham adrenalectomized rats. Furthermore, after central bombesin treatment adrenalectomized rats with corticosterone replacement became significantly more hypothermic than sham adrenalectomized rats. This implicates adrenal catecholamines, and possibly corticotrophin-releasing hormone, in modulation of this phenomenon.

Previous reports of bombesin-induced hypothermia at normal ambient temperatures have been associated with some type of pre-existing condition, which had to be present for hypothermia to be expressed. Early work by Avery
discovered bombesin-induced hypothermia in food-deprived rats tested at normal ambient temperatures incidentally, as it was not the main objective of their study. They hypothesized that bombesin may produce hypothermia in food-deprived rats secondary to a change in nutritional state, possibly due to a depletion of metabolic fuels (Avery & Calisher, 1982). This hypothesis appeared to spawn increased interest in altered nutritional conditions which might contribute to bombesin-induced hypothermia at normal ambient temperatures. Examination of possible metabolic suppression by bombesin revealed that in food-deprived rats there is no change in oxygen consumption associated with development of hypothermia at normal ambient temperatures (Babcock & Wunder, 1984). Babcock (Babcock & Barton, 1989; Babcock & Barton, 1990; Babcock, Barton, Gunion, & Rosenthal, 1992; Babcock, Barton, & Keene, 1989; Babcock, Barton, Perez, & Hearndon, 1989; Barton & Babcock, 1990), demonstrated that bombesin produces hypothermia in ad-libitum fed rats tested at normal ambient temperatures if first injected peripherally with insulin. He believed that peripheral injections of insulin simulated the fasted state by producing hypoglycemia and stimulating feeding. Later reports showed that bombesin-induced hypothermia could be exhibited in ad-libitum fed rats if first treated with
peripheral 2-deoxy-D-glucose (Babcock & Maisel, 1991) or if they had lesions of the ventromedial hypothalamus (Barton & Hawkins, 1993). Babcock has suggested that bombesin-induced hypothermia in ad-libitum fed animals treated with 2-deoxy-D-glucose may be secondary to a reduction of glucose utilization (Babcock & Maisel, 1991), or in the case of insulin treatment, glucose availability (Babcock & Barton, 1990). Superficially, this appears to be supported by findings that allowing animals to eat during testing attenuated bombesin-induced hypothermia (Barton & Babcock, 1990). Furthermore, examination of serum metabolic fuels seemed to reinforce this concept, as that serum glucose levels declined secondary to insulin treatment in ad-libitum fed rats made hypothermic with bombesin (Babcock, Barton, Gunion, & Rosenthal, 1992).

While the idea that reduced glucose availability or utilization may act in some way to allow bombesin to produce hypothermia, it seems inadequate in providing any concrete mechanism by which it would produce this event. York and Bray (personal communication), upon reviewing the findings of reports on bombesin-induced hypothermia at normal ambient temperatures, suggested an alternative mechanism through which the permissive events might influence this phenomenon. Their observation was, that all conditions which contribute to bombesin-induced hypothermia at normal ambient temperatures are also
associated with reduced sympathetic nervous system activation of thermogenesis. This observation became the nucleus for the development of the hypothesis that bombesin-induced hypothermia in animals tested at normal ambient temperatures is dependent upon a pre-existing state of suppressed sympathetic nervous system drive to brown adipose tissue. Previous studies provide significant support for this supposition. Acute starvation which permits bombesin-induced hypothermia (Babcock, Baker, & Moody, 1992; Babcock & Wunder, 1984; Calisher & Avery, 1984), also results in decreased norepinephrine turnover in brown adipose tissue (Dulloo, Young, & Landsberg, 1988; Landsberg & Young, 1978) as well as reductions in sympathetic nervous system drive (Sakaguchi, Arase, Fisler, & Bray, 1988). Treatment with 2-deoxy-D-glucose that allow bombesin-induced hypothermia (Babcock & Maisel, 1991), are also known to suppress sympathetic nervous system activity (Egawa, Yoshimatsu, & Bray, 1989; Landsberg & Krieger, 1989). Ad-libitum fed animals made hyperinsulinemic will become hypothermic following central bombesin (Babcock & Barton, 1989; Babcock & Barton, 1990; Babcock, Barton, Gunion, & Rosenthal, 1992; Barton & Babcock, 1990; Babcock, Barton, Perez, & Hearndon, 1989), and hyperinsulinemia also suppresses norepinephrine turnover in brown adipose tissue (Nishioka, Yoshida, Yoshioka, & Kondo, 1987).
Lesions of the ventromedial hypothalamus not only allow bombesin-induced hypothermia in ad-libitum fed rats (Barton & Hawkins, 1993), but are also known to suppress sympathetic efferent firing rates of nerves which innervate brown adipose tissue (Niijima, Rohner-Jeanrenaud, & Jeanrenaud, 1984). Furthermore, the act of feeding which attenuates bombesin-induced hypothermia (Barton & Babcock, 1990), also promotes heat production (Himms-Hagen, Hogan, & Zaror-Beherens, 1986; Rothwell & Stock, 1979; Stock & Rothwell, 1986) and increases firing rates of sympathetic nerves innervating brown adipose tissue (Sakaguchi, Arase, Fisler, & Bray, 1988). Thus, all conditions which promote bombesin-induced hypothermia are directly linked to suppression of sympathetic nervous system activation of thermogenesis. Additionally, those which attenuate bombesin-induced hypothermia are also linked to increased sympathetic nervous system activation of thermogenesis.

The potential modulatory effects on glucose utilization by adrenal catecholamines in bombesin-induced hypothermia is addressed by findings from Experiment IV. It is known that elevated adrenal epinephrine release limits glucose utilization by activation of α and β-adrenergic receptors (Cryer, 1992). Epinephrine activation of α-adrenergic receptors results in inhibition
of insulin release and promotes hyperglycemia (Clutter, Rizza, Gerich, & Cryer, 1988). In addition, activation of β-adrenergic receptors by epinephrine adds a synergism to this phenomenon by stimulating glucagon secretion, hepatic glycogenolysis, and gluconeogenesis (Skikama & Ui, 1975). Bombesin itself, produces marked increases in epinephrine after central administration (Brown, Carver & Fisher, 1988; Brown & Fisher, 1984). In addition, bombesin is known to produce hyperglucagonemia and hypoinsulinemia (Brown, Tache, & Fisher, 1979). Hyperglycemia following icv bombesin injection is eliminated by adrenalectomy, suggesting that bombesin acts through neural activation of the adrenals to produce this event. Given the data from Experiment IV, as well as these other findings, it appears that decreased glucose utilization is inadequate as an explanation for bombesin-induced hypothermia, especially in light of the fact that bombesin itself generates changes conducive to reduced glucose utilization. This is supported by findings that bombesin-induced hypothermia occurs in ad-libitum fed animals after peripheral injections of insulin, since insulin actually promotes increased glucose utilization (Cryer, 1992).

The data collected from the four experiments detailed here, along with previous findings provide overwhelming evidence that bombesin-induced hypothermia is mediated by sympathetic nervous system activity. This hypothesis is
efficaciously depicted in Figure 7. Bombesin acts on central sites, most likely the preoptic area, to stimulate peripheral vasodilation which results in heat loss. Under normal food-sated conditions, this does not result in hypothermia because of reflex activation of thermogenesis by the sympathetic nervous system. When animals are under conditions of acute starvation, peripheral injection of insulin or 2-deoxy-D-glucose, or have lesions of the ventromedial hypothalamus, the reflex sympathetic activation of thermogenesis is prevented at the level of the central nervous system, and hypothermia results. In bombesin-induced hypothermia in ad-libitum fed rats injected peripherally with chlorisondamine, the blockade of sympathetic activation of thermogenesis occurs at the level of the peripheral ganglia. When bombesin-induced hypothermia seen in food-deprived or chlorisondamine-treated rats is prevented by peripheral injection with CL-316,243, thermogenesis is restored secondary to direct stimulation of the $\beta_3$-adrenergic receptor in the brown adipose tissue.

Bombesin may have developed as an evolutionary process in homeotherms acting as an integrative mechanism to dissipate increased heat secondary to feeding related thermogenesis, and act as a satiety agent. The act of
Figure 7. Hypothesis that bombesin-induced hypothermia is dependant on suppression of sympathetic nervous system activity.
nutrient ingestion induces sympathetic nervous system activation of thermogenesis via activation of the brown adipose tissues (Himms-Hagen, Hogan, & Zaror-Behrens, 1986; Rothwell & Stock, 1979; Sakaguchi, Arase, Fisler, & Bray, 1988; Stock & Rothwell, 1986). It is known that ingestion of food stimulates release of the mammalian counterpart to bombesin, gastrin-releasing peptide, both in the periphery, and in the central nervous system (Kateb & Merali, 1992). Thus, it is possible that as a mammal consumes food, the gastrin-releasing peptide levels increase as does production. This may result in increased vasodilation and heat loss as a compensatory mechanism to thermogenesis. At the same time, it acts as a negative feedback signal to the actual consumption of food. In this event, hypothermia would not occur since sympathetic activation of thermogenesis is probably more than adequate to offset the heat loss caused by peripheral vasodilation. A simplistic pictorial representation of this theory can be seen in Figure 8.

Conclusions

1. Bombesin-induced hypothermia at normal ambient temperatures is dependent upon a pre-existing state of attenuated sympathetic nervous system activation of compensatory thermogenesis.
Figure 8. Theory of bombesin as an integrative peptide in coregulation of temperature and feeding.
2. Bombesin-induced hypothermia seen at normal ambient temperatures is not directly related to serum glucose levels associated with the fasted state alone.

3. Peripheral activation of thermogenesis, specifically in brown adipose tissue, prevents bombesin-induced hypothermia at normal ambient temperatures by restoring compensatory heat production.
REFERENCES


VITA

John Christopher Barton received the degree, Associate in Nursing, from Mobile College in May, 1978. Following Registration by the Alabama Board of Nursing, Mr. Barton was employed by Mobile Infirmary Medical Center in the Department of Surgery as an endoscopy specialist. In 1980 he completed training in Gastrointestinal Clinician Technology at the Carter Davis Memorial Gastrointestinal Diagnostic Laboratory, Emory University, Atlanta, Georgia. After completion of clinician training, Mr. Barton passed the certifying examination of the Council for Certification of Gastrointestinal Clinicians and was employed as Director of the Gastrointestinal Laboratory, Mobile Infirmary Medical Center.

In November, 1986, the candidate returned to the University of South Alabama where he received the Bachelors of Arts (1988) and Master of Science (1990) degrees in psychology. In 1990 he received The Outstanding Graduate Student Award from the Department of Psychology, as well as The Outstanding Graduate Research Award from the Alabama Psychological Association. In addition, Mr. Barton received a travel grant from the American Psychological Society as presenting author.

Mr. Barton entered the Graduate Program, Department of Psychology, Louisiana State University, August, 1990. The candidate has first authored 3 journal publications, 18
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DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Psychology

Title of Dissertation: Bombesin-Induced Hypothermia at Normal Ambient Temperatures: Contribution of the Sympathetic Nervous System

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