CMLS, Cell. Mol. Life Sci. 59 (2002) 272–287
1420-682X/02/020272-16 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Role of bombesin-related peptides in the mediation or integration of the stress response

Z. Merali[a,b,*], P. Kent[a] and H. Anisman[c]

[a] School of Psychology, University of Ottawa, 11 Marie Curie, Room 214 Vanier Hall, Ottawa, Ontario K1N 6N5 (Canada), Fax +1 613 562 5356, e-mail: merali@uottawa.ca
[b] Department of Cellular and Molecular Medicine, University of Ottawa, Ontario (Canada)
[c] Institute of Neuroscience, Carleton University, Ottawa, Ontario (Canada)

Received 16 July 2001; received after revision 27 August 2001; accepted 28 August 2001

Abstract. In addition to the relatively well established role of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) in the mediation of the stress response, there is reason to believe that bombesin-like peptides (BN-LPs) may also contribute to the mediation or integration of these responses and thus might be considered as putative 'stress peptides'. This review provides evidence supporting this contention by showing that (i) BN-LPs are present at brain sites known to be activated by stressors, (ii) stressor exposure alters utilization of BN-related peptides, (iii) exogenous BN administration mimics the endocrine, autonomic and/or behavioral effects elicited by stressors, and (iv) antagonism of BN action attenuates the behavioral and/or neurochemical effects of stressors or of exogenously administered peptide. The evidence presented also suggests that BN-LPs mediate their stress-relevant effects through activation of CRH and/or AVP neurons. Several hypothetical mechanisms for such peptidergic interactions are discussed as to the implications of considering BN-LPs as 'stress peptides'.

Key words. Neuroendocrine; pathology; neuromedin B; gastrin releasing peptide; stress.

Introduction

Stressful stimuli evoke a plethora of physiological and behavioral responses aimed at harm avoidance, blunting of the adverse impact of the stressor, and/or restoration of homeostasis [1–3]. The physiological stress response involves, among other things, activation of two interrelated systems, namely the sympathetic nervous system and the hypothalamic-pituitary adrenal (HPA) axis [4–6]. Sympathetic activation is reflected by the central release of norepinephrine (from widespread projections originating in the locus coeruleus) as well as the peripheral release of epinephrine and norepinephrine from the adrenal medulla [4, 7]. HPA activation is reflected by the cascading release of various secretagogues [including CRH and AVP] from the hypothalamus into portal circulation, ACTH from the anterior pituitary and finally corticosterone (or cortisol in humans) from the adrenal cortex [4, 6, 8, 9]. These systems can be activated by both systemic (e.g. infections, elevated blood pressure) and processive (e.g. psychological stimuli of emotional significance) stressors [8]. It is possible that these various types of stressors activate distinct neural circuits and/or transmitter systems, which eventually converge upon and activate CRH and/or AVP neurons in the parvocellular division of the paraventricular hypothalamic nucleus (PVN). Indeed, not only might neurogenic (physical) and psychogenic (psychological) stressors involve different processes, but there is also reason to believe that various types of processive (or systemic) stressors may also deploy distinct

* Corresponding author.

neural circuits. For instance, predator-related cues (e.g. predator odors) and conditioned (learned) fear responses may activate different aspects of the stress circuitry [10]. Although the role of classical neurotransmitters (e.g. norepinephrine, dopamine and serotonin) and peptides (e.g. CRH and AVP) in subserving the stress response have been firmly established, many other neuromodulators are also thought to contribute to the mediation and/or integration of these responses. It is our contention that bombesin-like peptides (BN-LPs), consisting of bombesin's mammalian counterparts, gastrin-releasing peptide (GRP) and neuromedin B (NMB), represent one class of neuromodulators that contribute to the integration and/or mediation of the stress response. This manuscript defines a set of four testable criteria that might serve to characterize or identify neuropeptides involved in the regulation of the stress response. This review will then assess experimental evidence, including some from our own laboratory, suggesting that BN-LPs meet the criteria defining a 'stress peptide', and may be involved in the mediation and/or integration of the stress response.

Characteristics of a stress peptide

In order to characterize a peptide as a 'stress peptide', several fundamental requirements ought to be met: (i) the putative 'stress peptide' should be present at brain sites known to be activated by stressors [e.g. as reflected by *c-fos* activation or 2-deoxy glucose (2-DG) accumulation following stressor exposure], (ii) exogenous peptide administration should mimic the endocrine, autonomic and/or behavioral effects elicited by stressors, (iii) antagonism of peptide action (e.g. using peptide receptor antagonists) should attenuate or block the behavioral and/or neurochemical effects of stressors or of exogenously administered peptide and (iv) stressor exposure should influence utilization of the peptide, as reflected by altered release, tissue levels, turnover [e.g. altered messenger RNA (mRNA) expression], or receptor function. These characteristics are by no means an exhaustive summary of all potential criteria, but rather represent a list of testable characteristics that can serve as a first step towards defining or characterizing stress peptides.

Of course, it is important to consider that the extent and possible direction of the peptidergic alteration may also be dependent upon the type of stressor to which an organism is exposed, as well as its intensity or duration. For instance, it was proposed that the neural pathways or circuits that are activated would vary as a function of the type of stressor to which an animal is exposed [8]. Stressors involving an immediate physiological threat (systemic stressor), such as an immune challenge or a respiratory stressor, may directly activate the PVN. In contrast, stressors which require interpretation by higher brain structures (processive stressor), such as psychological challenges, seem to first activate limbic forebrain structures (including the amygdala, hippocampus and prefrontal cortex) before filtering and transmitting relevant information to the PVN [8]. In addition to the type of stressor, a peptide's role in the stress response may change depending upon the duration and intensity of the stressor (acute vs. chronic; weak vs. strong) or as a function of individual organismic characteristics or experiential factors (genetic differences or life experiences) [1, 4]. Whereas these other factors are certainly relevant, the focus of this review will be on the four major characteristics presented earlier (see fig. 1).

BN-LPs

BN is a tetradecapeptide originally isolated from the skin of the European frog *Bombina bombina* [11]. Since its discovery, many BN-LPs have been isolated and subsequently divided into three subfamilies based on their carboxy-terminal tripeptide sequences. The three subfamilies include Bombesin, Ranatensin and Phyllolitorin. Currently, only two mammalian BN homologues have been identified. One is gastrin-releasing peptide $(GRP)_{1\text{-}27}$ (a 27-amino acid peptide sometimes referred to as mammalian BN), and the second is its decapeptide form, $GRP_{18\text{-}27}$ [often referred to as neuromedin C (NMC)] [12]. Neuromedin B (NMB) (in both its molecular forms, $NMB_{1\text{-}32}$, and $NMB_{23\text{-}32}$) belongs to the second or Ranatensin subfamily [13]. Receptors for BN-LPs have also been isolated and were subsequently localized in mammalian tissue. The first two receptors discovered were defined by their differential affinities for three BN agonists of mammalian origin. Thus, BB_1 receptors bind NMB with greater affinity than GRP, whereas BB_2 receptors bind GRP with higher affinity than NMB. More recently, new BN receptor subtypes (BB_3, and BB_4) have been identified; however, information pertaining to their functions is currently limited, as no known natural ligands have been shown to interact with high affinity to these receptors [3, 14].

Are BN-LPs present at stressor-responsive brain sites?

Mammalian BN-LPs are widely distributed throughout the CNS, as well as in peripheral structures, which are known to be responsive to stressful stimuli. Of the many brain regions activated by stressors, the parvocellular division of the PVN and the anterior and intermediate lobes of the pituitary (and hence the adrenal gland) appear to be particularly reactive in response to stressors, as reflected by marked increases of *c-fos* mRNA expression [15–19].

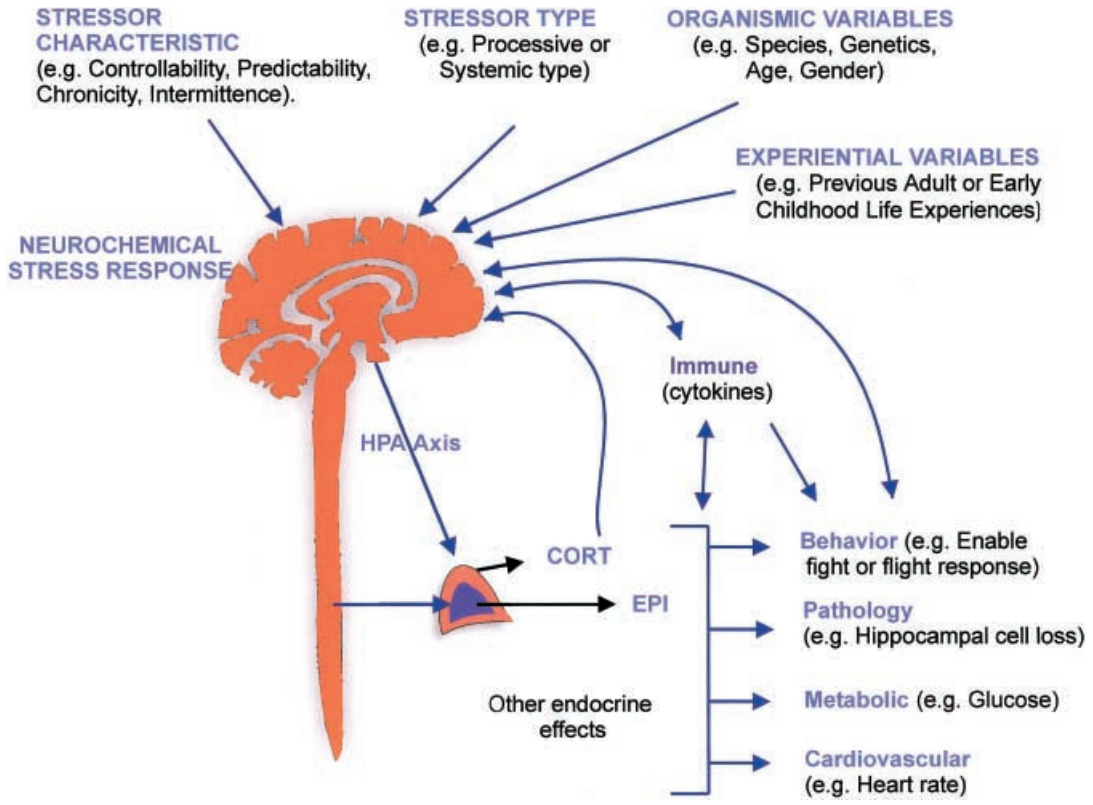

Figure 1. Factors that influence the neurochemical stress response. The response(s) to stressors are regulated by the central nervous system (CNS), and are influenced by a variety of factors including (i) the characteristics of the stressor (e.g. its controllability, predictability, chronicity and intermittence), (ii) the type of stressor [e.g. whether it is a perceived stressor (processive type; facing a predator, threatening environment) or whether it is a direct physiological insult (systemic type; loss of blood, infection) to the body], (iii) organismic factors (e.g. age, gender, genetic loading, species), (iv) experiential variables (e.g. early life experiences, particularly their history of stressor exposure) and (v) status of the immune system (whether it is activated and has released various cytokines). The CNS can promote physiological as well as behavioral responses which will allow the organism to deal with the actual or perceived threat to the organism. Such responses include the activation of the HPA axis (eventually leading to the release of glucocorticoids from the adrenal cortex) and/or the activation of the sympatho-adrenal system that leads to the release of catecholamines into the circulation. These types of responses can facilitate some of the other responses coordinated by the CNS, such as enabling the flight-or-fight response, increasing energy availability and ensuring adequate blood flow. However, when such responses become protracted, they can lead to pathology, such as hippocampal cell loss, ulceration of the gastrointestinal tract and so on.

As would be expected of stress-relevant peptides, immunoreactive (ir)-BN is present in all of these key elements of the HPA axis [20–23]. More specifically, GRP mRNA predominates within hypothalamic structures, including the parvocellular PVN, suprachiasmatic, supraoptic, preoptic and mammillary nuclei (as well, NMB mRNA has also been identified in some hypothalamic regions including the arcuate and supraoptic nuclei) [24, 25]. In contrast, NMB mRNA is more prominent than GRP mRNA at the anterior pituitary [26]. With respect to receptor distribution, high-to-moderate densities of BN-LP receptors are located at the PVN, anterior and arcuate hypothalamic nuclei, median eminence (ME) and anterior pituitary [27–30]. In situ hybridization studies have further revealed that BB_2 mRNA predominates within the hypothalamic nuclei, whereas both BB_2 and BB_1 receptor mRNAs are present at the anterior pituitary [25, 26, 31].

In addition to the elements of the HPA axis, ir-BN and BN-LP receptors are present in other CNS structures, which have been implicated in the stress response. For example, high levels of ir-BN are present in several caudal brainstem nuclei, including the nucleus of the solitary tract (NTS) and the parabrachial nucleus, as well as in several limbic structures, such as the bed nucleus of the stria terminalis (BNST), cortical and central amygdaloid nuclei (CeA), and the hippocampus, all of which have been associated with the stress response [20, 32, 33]. As would be expected from a stress peptide, GRP mRNA is prominent at all of the regions cited [24, 25]. In terms of BN-LP receptors, high-to-moderate densities are found within the amygdala, hippocampus, locus coeruleus, parabrachial nucleus and NTS [27–30]. Specifically, BB_2 receptor mRNA predominates in limbic structures, such as the amygdala and hippocampus, whereas both BB_1 and BB_2 receptor mRNAs are present in brainstem

structures such as the NTS [25, 31]. It is of interest that BB_1 receptors are also prominent at the dorsal raphé, and seem to regulate the release of serotonin at various forebrain regions [34]. Taken together, it appears that ir-BN mRNA expression and receptor density have been localized in many stress-relevant brain regions and might thus potentially contribute to both HPA and autonomic activity.

Does stressor exposure alter BN-LP release, levels and/or turnover, mRNA expression, and/or receptor function?

One approach to ascertain an association between BN-LPs and the stress response would be to assess whether stressor exposure functionally affected the endogenous system(s) utilizing BN-LPs. Specifically, does stressor exposure alter (i) the release of BN-related peptides, (ii) the tissue levels and/or turnover of these peptides, (iii) their mRNA expression and/or (iv) the receptor function of these peptides? Several experiments from our laboratory attempted to address such questions. Initially, we found that acute immobilization stress (10, 30 and 120 min) was associated with site-specific alterations of endogenous levels of BN-LPs at the hypothalamus and medulla and that the density of BN binding sites at the NTS, arcuate nucleus and PVN was altered in parallel (see figs. 2, 3) [35]. Next, we assessed whether the stress-related peptidergic alterations were dependent upon (i) the nature of the stressor, or (ii) genetic differences in stressor vulnerability. In two lines of rats that differed in anxiety response (comorbid with their differential seizure proneness; Fast and Slow seizing lines), acute exposure to a predator (i.e. ferret) or restraint induced alterations of ir-BN levels at the anterior hypothalamus and NTS, suggesting a functional relationship between this family of peptides and the stress response. Significantly, despite differences in the genetic makeup of the rat lines and the nature of the stressors employed, the regionally specific pattern of stress-related changes in the content of BN-LPs and the density of BN binding sites was quite similar. As will be discussed shortly, postmortem analyses for neuropeptide levels are likely not an adequate index of neuropeptide utilization. Thus, although these data do not provide an index of relative BN-LP activity in the two rat lines, the data are consistent with the view that stressors influence BN-LP activity at stressor-sensitive brain regions [36].

Several studies with higher anatomical resolution, using receptor autoradiography and brain micropunch, revealed that specific sites within the hypothalamus (such as the arcuate, paraventricular and anterior nuclei) and within the medulla (such as the NTS), may be involved in BN's mediation of the stress response [35]. These results are consistent with in situ hybridization findings showing a strong induction of immediate early gene expression at the NTS and numerous hypothalamic nuclei following exposure to a variety of different stressors [15, 37, 38]. It is notable that the brain micropunch and autoradiographic experiments did not reveal any stressor-induced alterations of ir-BN or BN binding sites at limbic structures, such as the amygdala or hippocampus. The stressors em-

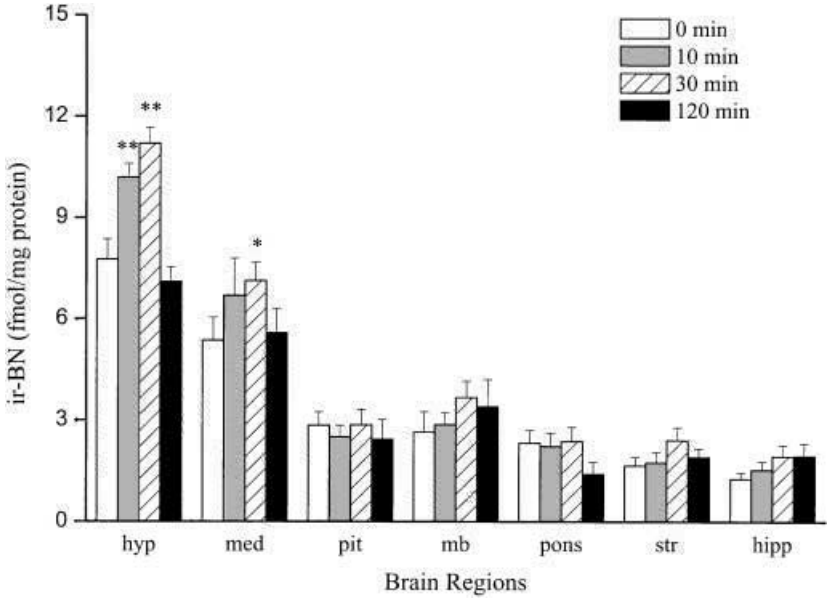

Figure 2. Content of ir-BN (mean ± SEM) in brain regions under nonstressed control condition and following exposure to 10, 30 and 120 min of immobilization. Brain regions include hypothalamus (hyp), medulla (med), pituitary (pit), midbrain (mb), pons, striatum (str) and hippocampus (hipp). *,** Significantly different from nonstressed control values at $P < 0.05$ and $P < 0.01$, respectively.

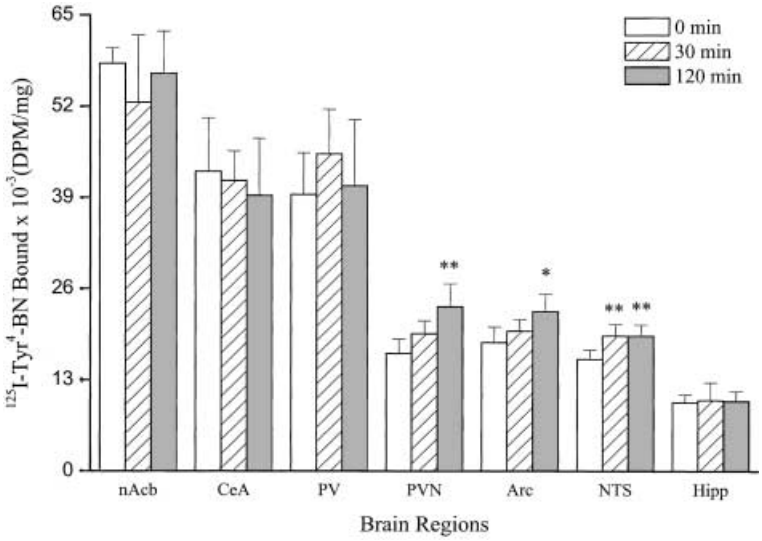

Figure 3. Density of BN binding sites in various brain regions under nonstressed control condition and following exposure to 30 and 120 min of immobilization. Brain regions assessed included the following: nucleus accumbens (nAcb), central amygdaloid nucleus (CeA), paraventricular thalamic nucleus (PV), paraventricular hypothalamic nucleus (PVN), arcuate nucleus (Arc), nucleus of the solitary tract (NTS) and hippocampus (Hipp). Results are expressed in DPM/mg as mean ± SEM *, ** Significantly different from non-stressed control values at $P < 0.05$ and $P < 0.01$, respectively.

ployed in these experiments (immobilization/restraint and ferret exposure) are considered psychological or 'processive' in nature, and are thought to require significant sensory processing by the limbic structures in the brain in order to take on physiologic meaning [8]. It is possible that BN-LPs are involved with generalized reactivity to stressors (involving direct HPA axis activation) as opposed to higher-order interpretation of the stressor (which would involve activation of limbic brain structures). In contrast to the absence of altered BN-LPs at the limbic structures, we observed significant fluctuations of ir-CRH content and the endogenous release of CRH at the CeA following restraint stress and ferret exposure [36]. Based on these results, it would seem that BN-LPs and CRH play distinctive roles in the stress response, with CRH being more involved with higher-order sensory processing in addition to direct HPA activation. This interpretation, however, was considered to be highly provisional, particularly as the in vivo release of BN-LPs from the CeA was not assessed. Although no change of ir-BN at the CeA was observed, it is conceivable that this 'snap shot' assessment of postmortem tissue peptide levels may have failed to detect changes of turnover, if for instance the alterations of peptide utilization were offset by compensatory changes in peptide synthesis or degradation. Indeed, in an in vivo microdialysis study, we observed that acute restraint stress elicited the release of both CRH and BN-LPs from the CeA [39]. Thus, it appears that these peptides may be involved in the physiological manifestation of the stress response and that the lack of change in the tissue level of peptide (post-mortem) does not necessarily reflect lack of effect on the peptide utilization [40].

Taken together, it appears that exposure to a variety of different stressors can elicit site-specific changes of endogenous levels of BN-LPs. Although these results provide some indication of key structures where BN-LPs might be acting to mediate, or modulate the stress response, they do not provide insight into the underlying mechanism(s) of action. This is especially true since alterations in postmortem tissue content (or lack thereof) are difficult to interpret. For example, increased tissue peptide levels of ir-BN may reflect (i) an increased synthesis of the peptide and/or (ii) decreased release of the peptide or (iii) increased peptide release accompanied by even greater rate of its synthesis. Similarly, decreased tissue levels of ir-BN may indicate (i) decreased synthesis and/or (ii) increased release, or (iii) increased synthesis accompanied by an even greater rate of release. The failure to detect fluctuations in tissue peptide levels may be similarly ambiguous, as it could reflect either no change in the rate of synthesis and/or release or, alternatively, a rate of release that is matched by an increased rate of peptide synthesis. Due to limitations in the interpretation of regional peptide level changes, we attempted to assess the dynamic in vivo release of BN-LPs (GRP and NMB) from the anterior pituitary in response to stressor exposure, using the push-pull perfusion technique [40]. In addition, release of other stress-relevant peptides, including CRH and AVP, were monitored. We assessed the anterior pituitary in these studies because it is an integral component of the HPA axis, being the primary target of peptides

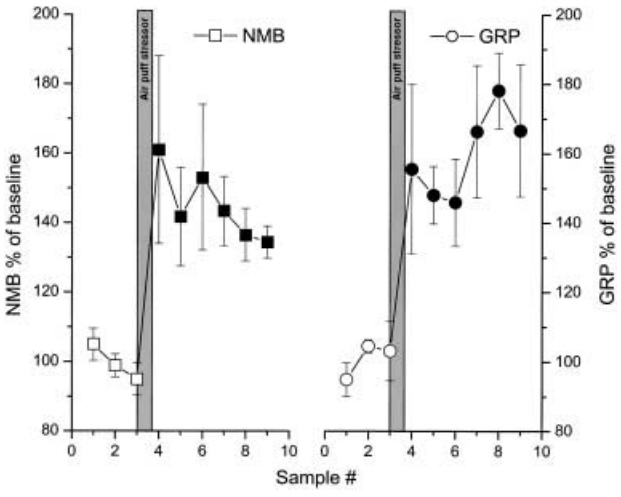

Figure 4. Interstitial levels of GRP and NMB (expressed as a percentage of the baseline) at the anterior pituitary, following exposure to an acute stressor (air puff). Solid symbols represent points that are significantly different from the last baseline sample of respective treatment conditions.

released from the median eminence-arcuate complex (Me/Arc), and the source of ACTH secretion during the stress response. In addition, in a recent micropunch experiment, we found striking alterations of ir-BN at the pituitary in response to audiogenic stressor exposure (unpublished observations). It was observed that exposure to an air-puff stressor (over a 5-min period; 1 pulse/min) significantly increased interstitial levels of both GRP and NMB (as well as CRH and AVP) at the anterior pituitary (see fig. 4). Although the stressor-induced increase of peptidergic levels at the anterior pituitary may reflect local release (at the anterior pituitary), it is more likely that it represents peptide release upstream (at the Me/Arc). Indeed, we have recently shown a tight temporal association between peptidergic release from the Me/Arc and availability of those peptides at the anterior pituitary [40]. These findings suggest that like CRH and AVP, BN-LPs are released from the Me/Arc in response to stressor exposure.

Does exogenous administration of BN mimic the endocrine, autonomic and behavioral effects elicited by stressors, and are these effects blocked or attenuated by the blockade of BB_1, or BB_2 receptors?

Several laboratories, including ours, have shown that BN, along with its mammalian counterparts, GRP and NMB, can potently stimulate the release of ACTH from the anterior pituitary and corticosterone from the adrenal cortex [41–47]. It is apparent that the ability of GRP to stimulate ACTH and corticosterone release is mediated via BB_2 receptors, as central administration of a competitive and specific BB_2 receptor antagonist completely blocked the GRP-induced increases in plasma ACTH and corticosterone levels [43]. The available data have been somewhat less consistent with respect to BN-induced endocrine effects following peripheral administration of the agonists. For instance, both Olsen et al. [44] and Garrido et al. [43] found that intravenous injection of GRP did not affect plasma ACTH or corticosterone levels in rats. In contrast, others reported that intravenous infusion or intraperitoneal administration of GRP or BN significantly elevated plasma ACTH and corticosterone levels in rats and dogs [48–50]. In addition, in normal men intravenous infusion of GRP stimulated the release of ACTH, β-endorphin and cortisol [51]. It has been suggested that the discrepancies concerning the endocrine results following peripheral administration of BN-LPs may be related to species-dependent differences in sensitivities to BN-LPs, or differences in the dosages of BN-LPs utilized [44]. Indeed, in most studies where systemically administered BN-LPs have been found to stimulate ACTH and corticosterone (or cortisol) levels, the doses of the peptide(s) have typically been higher (>2100 pmol) than in the studies where such a stimulatory effect was not seen (typically <700 pmol) [43, 44, 48]. The inconsistent results obtained with systemic BN administration, as well as the need for relatively high doses of the peptide in order to provoke a stimulatory effect on ACTH or corticosterone secretion, has led to the suggestion that BN-LPs may be acting predominantly through central mechanisms at suprapituitary sites, such as the PVN or ME, to stimulate the HPA axis. In support of this contention, Gunion et al. [45] demonstrated that infusion of BN into the PVN significantly increased blood corticosterone levels. In addition, Garrido et al. [42] demonstrated that low-to-moderate concentrations of GRP (1 and 10 nM) stimulated the release of CRH-like material from the hypothalamus, whereas high concentrations of the peptide (100 and 1000 nM) were needed to stimulate the release of ACTH and corticosterone from the anterior pituitary and adrenal cortex, respectively. The effects of GRP at the hypothalamus, anterior pituitary and adrenals appear to be mediated via BB_2 receptors, as the BB_2 receptor antagonist (Leu^{13}-ψ-CH_2-NH-LEU^{14}) BN blocked GRP's efforts on hormone secretion from these sites [42]. However, firm conclusions await the availability of highly selective and specific BB_1 receptor antagonists.

As mentioned earlier, BN-LPs effectively influence sympathetic activity. In particular, central BN administration dose-dependently increased plasma levels of epinephrine and norepinephrine [52–55] and increased plasma glucose levels [52, 56–58]. The ability of BN to increase levels of plasma glucose is dependent on adrenal epinephrine secretion, which diminishes plasma insulin levels and increases plasma glucagon levels [52]. Although the underlying mechanisms mediating BN's sympathoad-

renomedullary functions are currently not known, direct electrophysiological evidence indicated that centrally applied BN elevates the activity of both sympathetic and adrenal branches of splanchnic nerves, suggesting that BN has a direct effect on sympathetic outflow [59]. Furthermore, hypophysectomy did not prevent BN-induced hyperglycemia, and adrenalectomy did not prevent the BN-induced rise in plasma concentrations of norepinephrine [52, 53]. These findings suggest that BN's involvement in autonomic activation is centrally mediated and independent of the HPA axis. Moreover, BN-induced elevations of plasma epinephrine levels are not blocked by central administration of dopaminergic, adrenergic and cholinergic antagonists [53]. To determine the relevant central site(s) of action, this peptide was microinjected into various brain sites of unanesthetized rats [53, 54]. Injection of BN into the NTS produced a dramatic elevation of circulating plasma catecholamine levels relative to its effects at other brain sites. Iguchi et al. [57] also found that microinjection of BN into the ventromedial and lateral hypothalamic nuclei resulted in a marked increase of plasma glucose levels, raising the possibility that these sites also play a role in mediating BN-induced hyperglycemia. Additional support for a role for BN-LPs in autonomic activation is provided by the finding that central BN administration significantly increased mean arterial pressure [54, 60]. It has been suggested that this action is secondary to the increase of plasma epinephrine levels. Consistent with this contention, acute adrenalectomy or pretreatment with the α-adrenergic receptor antagonist phentolamine blocked the increase of mean arterial pressure induced by BN injection [60].

In addition to the neurochemical and endocrine findings, there is also behavioral support for the notion that BN-LPs might qualify as stress peptides. Unfortunately, as the potential involvement of BN-LPs in the stress response is a relatively new formulation, the behavioral data supporting this contention are limited. For instance, data are not available regarding the effects of BN or its antagonists on several traditional tests of anxiety, such as the elevated plus maze, light-dark box test, or the startle and fear-potentiated-startle responses. However, in behavioral tests that have been conducted, central or systemic administration of BN-LPs induced behavioral effects resembling those elicited by stressor exposure, or central CRH administration [61–65].

One of the characteristic behavioral responses to stressors is to reduce food consumption, coupled with weight loss [66]. In a similar fashion, both peripheral and central administration of BN and its mammalian counterparts (GRP and NMB) dose-dependently suppress food intake in a variety of species [62, 67–69]. Indeed, the literature is replete with evidence supporting the notion that BN-LPs may represent physiologically relevant mediators of satiety. However, the current tenet suggesting that these peptides mediate stress response is not mutually exclusive of that suggesting that these peptides mediate satiety. Indeed, it is possible that stressor-induced suppression of food intake may recruit the very same circuitry or signaling mechanism(s) that mediates satiety. In other words, under normal physiological conditions, the satiety-signaling system utilizing BN-LPs may operate independently; however, stressor-associated suppression of food intake would require activation of that system. Although direct evidence supporting this contention is lacking, we have shown that pretreatment with the CRH receptor antagonist αh-CRF, which inhibits stressor-induced suppression of ingestion [66], blocked BN-induced suppression of food intake [70]. It is of interest that many of the brain regions activated during food ingestion are also activated upon stressor or BN exposure [71], including several hypothalamic nuclei (paraventricular, arcuate and dorsomedial nuclei). Furthermore, in vivo or physiological release of BN-LPs from the PVN has been shown to occur in a meal-dependent manner [72, 73]. In addition, microinjection of exquisitely low doses of BN-like peptides into the NTS suppressed food intake [63] and lesions to the area postrema and NTS blocked the ability of either central or systemic BN to inhibit feeding [74, 75]. Thus, like stressor exposure, it appears that BN-LPs suppress food intake, and the possibility exists that stressor-induced suppression of ingestion is induced by the stimulation of endogenous BN-related system(s) involved in the physiological regulation of food intake.

Animals, particularly rodents, often engage in grooming behavior when confronted with stressful conditions. It is thought that this 'displacement' behavior may be a coping style aimed at dealing with the stressor [76–78]. As such, it is interesting that centrally administered BN or GRP increases grooming behavior [61, 63, 79, 80]. Since peripheral BN injection was without such effect, central mechanisms are implicated in the mediation of this BN-elicited behavior. Although the central mechanisms are currently not known, there is evidence of involvement of both the dopaminergic and cholinergic systems, as pretreatment with either a selective D_1 receptor antagonist or a muscarinic receptor antagonist can attenuate BN-induced grooming [81, 82]. Interestingly, Van Wimersma et al. [83] examined in detail the elements that comprise BN-induced grooming (of which the main component is scratching) and found that pretreatment with haloperidol induced a general reduction of BN-induced grooming, whereas pretreatment with naloxone suppressed the scratching element of BN-induced grooming behavior. These findings suggest that the opioid system may be involved in the display of the scratching element of BN-induced grooming, whereas the dopaminergic system appears to be involved in other elements of BN-induced grooming. Parenthetically, neither adrenalectomy nor hypophysectomy prevented the excessive grooming induced

by BN administration, suggesting that these BN-induced effects are independent of the HPA axis [79].

In addition to grooming, a characteristic behavioral response to stressors among rodents is that of reduced exploration in a novel environment. In a familiar environment, however, mild stressors may provoke increased exploration. Once again, BN-LPs influence exploratory behavior in a situation-specific fashion. Several investigators have shown that central administration of BN dose-dependently enhanced locomotor activity in rats tested in a familiar environment [28, 84, 85]. In contrast, reduced exploration is apparent when BN-LPs are administered in a novel (presumably stressful) environment. In this context, Itoh et al. [64] demonstrated that central administration of BN, NMB, GRP and other related peptides induced a marked reduction of locomotion and rearing in rats placed in a novel open field. Again, these BN-induced behavioral effects are consistent with those observed in response to stressors. There is evidence that the ability of BN to enhance locomotor activity is mediated, at least in part, via dopaminergic neurons, as pretreatment with dopamine receptor antagonists attenuated this BN-induced effect [84, 85].

Just as locomotor activity is situation dependent, we have observed that the consumption of a highly palatable snack (granulated sucrose) also varies as a function of the environmental context. When these snacks are offered to rats in a familiar environment (e.g. home cage), they readily consume the snack. In an unfamiliar environment (e.g. when the cage, or the bedding is changed) latency to initiate consumption is markedly increased, whereas the amount consumed is reduced [86, 87]. It is of particular interest that anxiolytic treatments resulted in an increase of snack consumption in the novel cage but not in the home-cage environment, suggesting that novelty-induced suppression of food intake is related to anxiety generated by the new environment. As would be expected, the neuromedin B antagonist (PD 176252) was found to attenuate the effects of the novel environment, but not in the home cage (see fig. 5). Although this effect may be attributible to neuromedin B receptor (BB_1) blockade, this conclusion remains tenuous, as PD 176252 also has affinity (although about an order of magnitude lower) for GRP (or BB_2) receptors [88, 89]

Finally, just as stressor exposure induces an antinociceptive effect [90, 91], exogenous BN administration has also been shown to produce antinocioception in rats. Pert et al. [28] demonstrated that microinjection of BN into the periaqueductal gray matter produced an antinocioceptive reaction in both the hot plate and tail flick tests. These effects were not blocked by naloxone administration, suggesting that the opioid system is not involved in mediating this effect of BN.

Central mechanisms mediating stress-related effects of BN-LPs

Although the neuronal mechanisms underlying the stress-relevant BN-induced effects remain to be fully elucidated, there is evidence that BN-LPs stimulate the HPA axis by

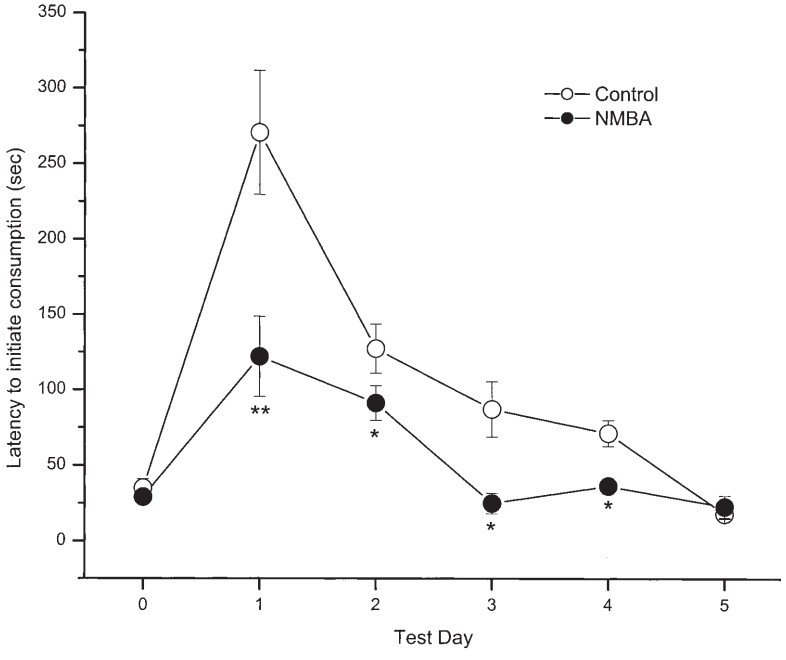

Figure 5. Anxiolytic effect of BB_1/BB_2 receptor antagonist: attenuation of novel environment induced increase in latency to initiate snack consumption (mean ± SEM) in rats that received central injections of either the antagonist or vehicle (control). *, ** Significantly different from saline values at $P < 0.05$ or < 0.01, respectively.

acting through other ACTH secretagogues, in particular CRH and/or AVP. In support of this contention, we have shown that blockade of central CRH receptors with αh-CRF attenuated BN-induced endocrine (elevations in plasma ACTH and corticosterone levels) and autonomic (elevations in plasma norepinephrine, epinephrine and glucose levels) effects [92] (see fig. 6). In an initial experiment the central administration of CRH antagonist, αh-

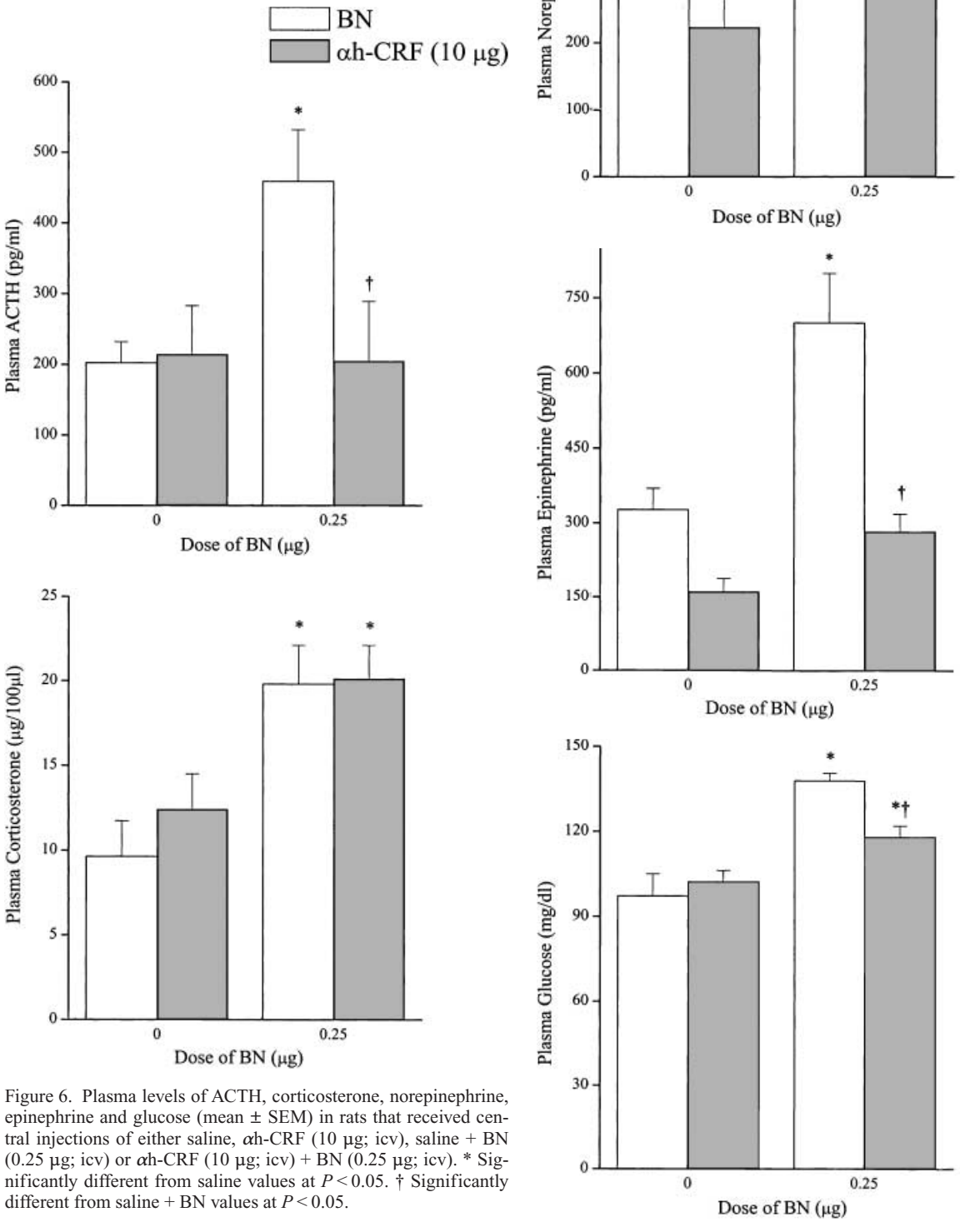

Figure 6. Plasma levels of ACTH, corticosterone, norepinephrine, epinephrine and glucose (mean ± SEM) in rats that received central injections of either saline, αh-CRF (10 µg; icv), saline + BN (0.25 µg; icv) or αh-CRF (10 µg; icv) + BN (0.25 µg; icv). * Significantly different from saline values at $P < 0.05$. † Significantly different from saline + BN values at $P < 0.05$.

CRF, attenuated a variety of effects elicited by BN, including circulating levels of glucose and epinephrine. However, this treatment did not affect the BN-induced elevation of plasma corticosterone levels (measured at a single 30-min time point). The possibility was considered, however, that the absence of an effect on corticosterone might have been due to the doses used or the time of sampling. Thus, in a second experiment, a slightly lower dose of BN was used (0.2 µg; icv), and blood samples were collected at different time points to assess the time-dependent changes in blood corticosterone levels (see fig. 7). Using these parameters, pretreatment with αh-CRF attenuated the BN-induced rise in blood corticosterone levels [92]. In a similar fashion, Olsen et al. [44] previously demonstrated that central GRP administration dose-dependently stimulated ACTH release and potentiated the ACTH-stimulating effects of CRH and AVP. In addition, pretreatment with either CRH or AVP antisera blocked GRP-induced ACTH release by 60%, whereas pretreatment with combined antisera completely blocked this effect. It has also been shown that central administration of αh-CRF blocked the GRP-induced increase of ACTH and corticosterone [43]. In addition, in vitro evidence indicated that BN-LPs may be acting via CRH neurons. For example, using isolated perfused anterior pituitary cells, either BN or GRP enhanced the CRH-induced ACTH release [93]. Moreover, Au et al. [41] showed that administration of GRP or BN dose-dependently potentiated CRH-induced ACTH release from mixed anterior pituitary cell cultures. This effect was completely blocked by the BB_2 receptor antagonist D-Tyr^6 BN(6–13) propylamide, suggesting that BN-LPs exert their effects by binding to BB_2 receptors on anterior pituitary cells.

As it appeared that BN-LPs might be mediating their effects through central CRH and/or AVP neurons, it was of interest to determine the anatomical locus (loci) for these peptidergic interactions. An initial mapping study in our laboratory (using brain micropunch approach) revealed that central BN administration (0.25 and 0.5 µg; icv) influenced the levels of CRH and AVP at various hypothalamic and extrahypothalamic brain regions, many of which are thought to play a role in the stress response. Specifically, central BN administration significantly decreased endogenous levels of CRH at the ventromedial and anterior hypothalamic nuclei (VMH, AH), NTS and CeA. In contrast, BN administration was associated with a significant increase in the endogenous levels of AVP at the PVN and Me/Arc and a significant decrease at the VMH [94]. In order to assess the functional relevance of these observed tissue level alterations, more direct push-pull perfusion experiments were performed to assess the dynamics of the in vivo release of CRH and AVP from the Me/Arc (being the primary source of CRH release during HPA activation) and downstream at the anterior pituitary

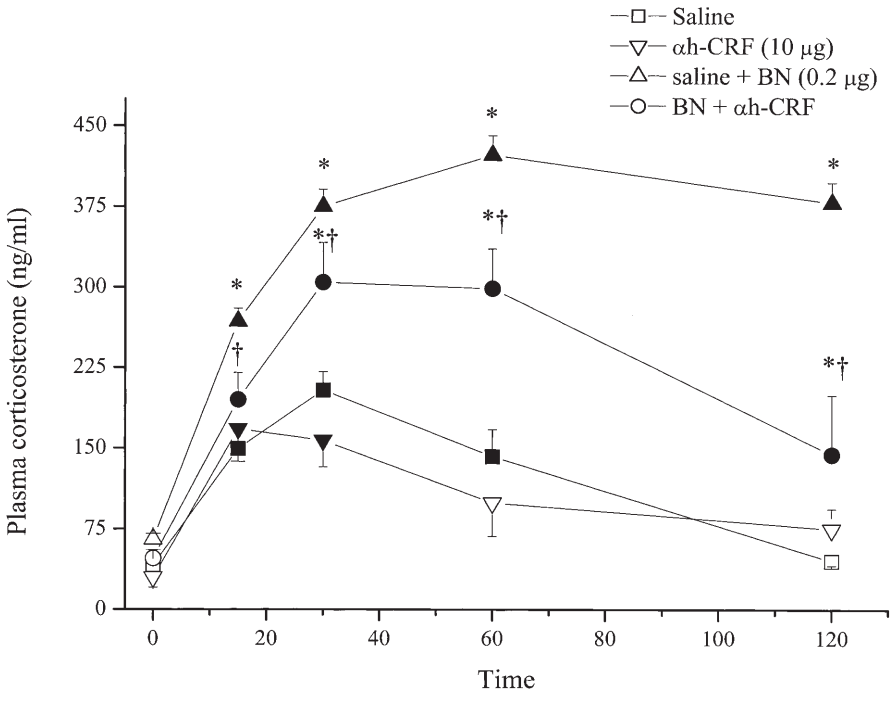

Figure 7. Plasma concentrations of corticosterone (mean ± SEM) at the 0, 15, 30, 60 and 120 min time intervals (following drug administration) in rats that received central injections of either vehicle (saline), αh-CRF (10 µg; icv), saline + BN (0.2 µg; icv) or αh-CRF (10 µg; icv) + BN (0.2 µg; icv). Closed symbols represent points that are significantly different from (within-treatment condition) baseline values at $P < 0.05$. * Significantly different from (between-treatment condition) time-point matched saline values at $P < 0.05$. † Significantly different from (between-treatment condition) time-point matched saline + BN values at $P < 0.05$.

(where these peptidergic messages influence ACTH release). It was observed that central BN administration stimulated the release of CRH and AVP from the Me/Arc, translating into an increased availability of these ACTH secretagogues at the anterior pituitary (see figs. 8 and 9).

Potential sites and mechanism(s) of action

Collectively, the available data provide support for the contention that BN-LPs mediate their effects, at least in part, via site-specific CRH and/or AVP neurons. Many questions remain unanswered with respect to the precise nature of these peptidergic interactions. In fact, several potential sites of action could be proposed to explain our pharmacological findings. These potential modalities of action are not mutually exclusive. In fact, it has become increasingly more apparent that peptides often function in an overlapping and redundant manner [95]. Just as there appear to be multiple peptides capable of performing similar tasks, it is plausible that a single peptide is able to perform its functions through multiple neuronal mechanisms. This kind of overlap and redundancy would help to ensure the functioning of a psychological fail-safe system.

The first and probably most obvious mechanism by which CRH- and/or AVP-expressing neurons are affected is via BN-LP receptors (BB_1 and/or BB_2) located on the cell bodies and/or dendrites of CRH and/or AVP neurons. Following stressor exposure (or exogenous BN or related peptide administration), BN-LPs would bind to these specific receptors to provoke the release of CRH and/or AVP. It is well established that CRH-containing neurons in the parvocellular division of the PVN (that coexpress AVP) comprise the final common pathway controlling the activity of the HPA axis [8]. Given that central BN administration elicits the release of CRH and AVP from the Me/Arc, a region where CRH neurons originating from the PVN terminate, it is likely that BN-LPs exert an effect

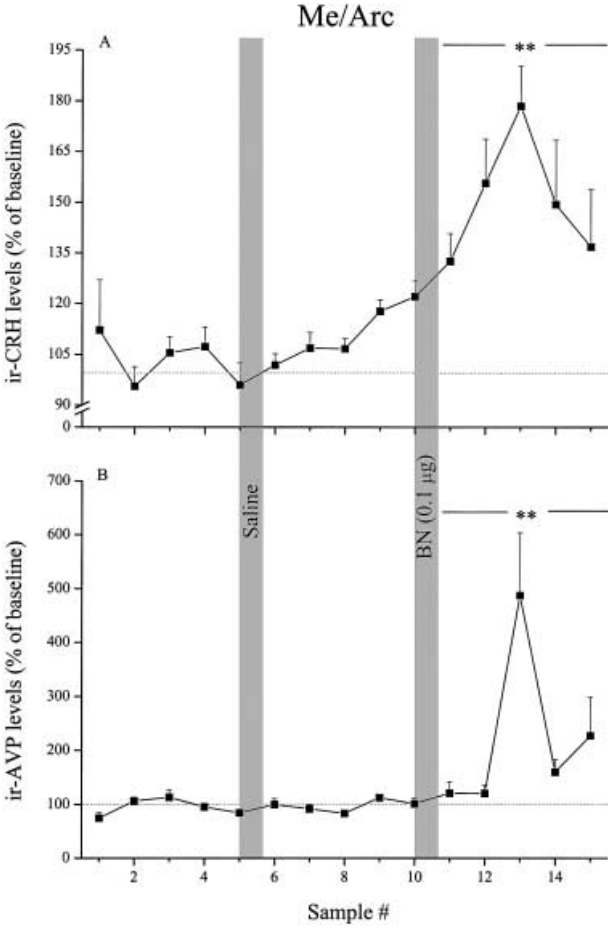

Figure 8. Levels of CRH and AVP at the Me/Arc under baseline condition, and following central administration of saline and BN (0.1 µg; icv). The basal values for each subject were averaged over the five baseline samples and defined as 100%. All values were then expressed as a percentage of that baseline. ** Significantly different from baseline condition at $P < 0.01$.

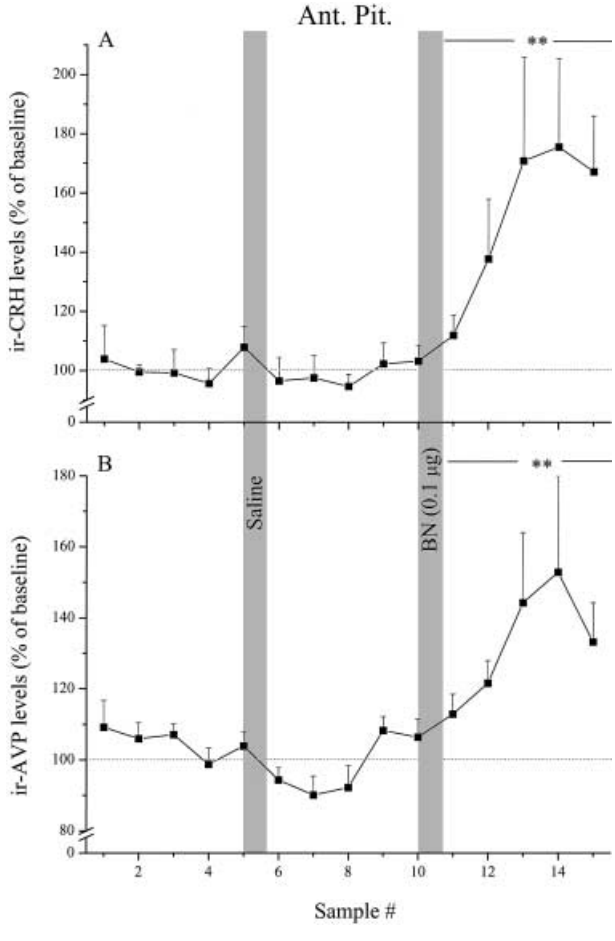

Figure 9. Levels of CRH and AVP at the anterior pituitary under baseline condition, and following central administration of saline and BN (0.1 µg; icv). The basal values for each subject were averaged over the five baseline samples and defined as 100%. All values were then expressed as a percentage of that baseline. ** Significantly different from baseline condition at $P < 0.01$.

on the CRH-containing neurons at the PVN. This interaction could occur directly, through local release of BN-LPs at the PVN, or through release originating from more distal BN-LP projections. Indeed, diverse sets of inputs arise from brainstem, limbic and/or hypothalamic pathways to converge on the parvocellular CRH-expressing neurons [8, 96]. Like noradrenergic and adrenergic neurons that project from the NTS to synapse on the cell bodies and dendrites of CRH-containing neurons at the PVN [97, 98], BN-LP neurons may also extend from brainstem structures such as the NTS to the PVN. Although tract-tracing studies have revealed BN-LP fibers projecting from the PVN to the dorsal vagal complex (which is encompassed within the boundaries of the NTS), BN-LP neurons extending in the opposite direction have not yet been identified [99, 100]. Interestingly, BN-LPs are co-localized with tyrosine hydroxylase (the rate-limiting enzyme in the biosynthesis of norepinephrine) at the NTS [99]. This might indicate that BN-LPs may be cosecreted with norepinephrine and/or epinephrine at the PVN to stimulate CRH release.

As an alternative to the aforementioned hypothesis, it is possible that BN-LPs may activate CRH neurons in the PVN via indirect routes. Consistent with this notion, direct microinjection of BN into the NTS stimulates an increase of circulating plasma norepinephrine and epinephrine levels [54]. Thus, it remains likely that BN-LP neurons may synapse on catecholaminergic neurons of the NTS that project to the PVN. This does not exclude the possibility that BN-LP neurons synapse on CRH- and/or AVP-expressing neurons directly at the NTS. The observation that central BN administration significantly decreases ir-CRH content at the NTS is in keeping with this notion. Activation of CRH neurons at the NTS may then stimulate the HPA axis, either directly through their own projections to the PVN, or indirectly through noradrenergic or adrenergic fibers. Aside from the NTS, it is possible that BN-LP neurons projecting from other stress-relevant limbic structures such as the CeA, hippocampus and/or BNST or hypothalamic nuclei (ventromedial, anterior and/or arcuate nuclei) synapse with parvocellular CRH-expressing neurons in the PVN. Although there is only limited information with respect to the location of BN-LP projections, moderate to high levels of ir-BN and BN binding sites are present at most of the aforementioned limbic and hypothalamic structures [20, 27, 32]. It is also possible that BN-LP neurons may synapse directly on CRH- and/or AVP-expressing neurons (or on neurons expressing other stress-relevant neurotransmitters or neuromodulators) at these various limbic and/or hypothalamic sites. This notion is consistent with our findings of decreased ir-CRH content at the CeA and anterior hypothalamus and a decrease in both ir-CRH and ir-AVP content at the ventromedial hypothalamus following central BN administration [40]. Moreover, as indicated earlier, we have demonstrated that exposure to restraint stress produced a pronounced increase of CRH and BN-LP release at the CeA [39]. Irrespective of where BN-LPs bind to specific receptors on CRH and/or AVP neurons, electron-microscopic studies are needed to analyze possible synaptic contacts between BN-LP axons and CRH-positive dendrites.

Simplistically, it might be assumed that the relationship between BN-LPs, CRH and AVP is unidirectional; i.e. BN-LPs cause the release of CRH and/or AVP and not vice versa. Although this assumption needs to be further investigated, it is not in keeping with the contention that peptides generally function in an overlapping and redundant capacity. This said, it is more likely that the relationship between BN-LPs, CRH and AVP is bidirectional. The next two models proposed incorporate this concept. The first is based on the relationship between CRH and AVP. It is well established that most immunodetectable AVP in the external zone of the ME is found in CRH-containing terminals, implying that AVP is coproduced in the CRH-expressing neurons of the PVN [101]. Like AVP, BN-LPs could potentially be coproduced in parvocellular CRH-expressing neurons and costored with CRH in the nerve terminals of the external zone of the ME. Indeed, there is some evidence that this in fact is the case, as co-localization of BN-LPs and CRH has been observed in the ovine ME [21]. Although the basic principle of the first model, namely that BN-LP receptors are located on CRH neurons, would still hold true for this second model, the difference is that when BN-LPs bind to their receptors, CRH, AVP or BN-LPs would be released. Similarly, if CRH binds to specific receptors on CRH neurons co-storing AVP and/or BN-LPs, then CRH, AVP and/or BN-LPs would be released. Evidence for or against this model could be provided by further characterization of the extent of colocalization of CRH and BN-LPs at the ME (as well as in other regions) across species. In addition, further studies characterizing the in vivo (or in vitro) peptidergic release from the ME are needed. Although we demonstrated (using push-pull perfusion) increased availability of CRH, AVP, GRP and NMB at the anterior pituitary (located downstream from the ME) in response to air-puff exposure, the temporal resolution in this method is not sensitive enough to provide a clear indication as to which of the peptides are coreleased from the external zone of the ME.

Finally, the possibility should be considered, as indicated earlier, that BN-LPs and CRH (and/or AVP) function as cooperative and interdependent parallel systems. This model is similar to one proposed by Cooper and Dourish to explain pharmacological interactions between cholecystokinin (CCK) and 5-HT in the regulation of satiety [102]. In this model, the relationship between CCK and 5-HT is cooperative in nature, as the release of one would enhance the release of the other and vice versa. Moreover,

according to this model, CCK and 5-HT can act in parallel (i.e. they perform separate actions at distinct receptor sites) or interdependently (i.e. both systems are necessary for the full expression of their effects, because the receptors are interfaced through a common gate). Thus, blockade of one system will affect the ability of the other to produce its effect. Although we have shown that BN-LPs cause the release of CRH, we have not assessed whether CRH can elicit the release of BN-LPs. This observation would be necessary in order to satisfy the assumption that the relationship between BN-LPs and CRH is cooperative. Moreover, although we have shown that blockade of central CRH receptors attenuates BN-induced HPA and sympathetic activation, as well as BN-induced behavioral effects [70], studies on the effects of BN receptor blockade on CRH-induced endocrine, autonomic and behavioral effects are currently lacking. Again, observations along these lines are necessary to satisfy the interdependence assumption.

Implications

The present review suggests that the BN family of peptides ought to be recognized as 'stress peptides'. This designation for BN-LPs has several potential neuroscientific and heuristic implications. Although a normal stress response is largely viewed as adaptive and crucial for the maintenance of mental and physical well being, dysregulation of the stress response system(s) is thought to culminate in pathophysiology. In this respect, a large body of evidence has emerged linking HPA axis overactivity to a host of pathological conditions including psychiatric disorders (i.e. major depression and anxiety disorders), cardiovascular disease, gastrointestinal dysfunction, suppression of growth, immune system dysfunction and selective brain cell damage as well as in eating disorders like anorexia nervosa [103, 104]. This may be particularly pertinent to the situations where stressors impact on ingestive behavior. As we saw earlier, the peptidergic alterations as well as responses to exogenously administered peptides is often situation specific and influenced by stress history, factors currently being implicated in certain forms of eating disorders [1, 4, 9]. Thus, as in the case of CRH and AVP [105–110], it is likely that BN-related peptides may also be associated with stress-related pathology. Indeed, we have recently obtained evidence from two additional lines of inquiry further affirming the importance of this family of peptides in stress-response: (i) in a study of brains from suicide victims and matched controls, we observed site-specific alterations in the endogenous levels of CRH, AVP and BN-LPs [unpublished observations], and (ii) in rodents, a mixed BB_1/BB_2 receptor antagonist was shown to have potent antianxiety effects, which may be associated with suppression of serotonin release at forebrain sites [Andrews et al., personal communication]. Thus the expansion of the sphere of stress peptides to include the BN family of peptides could open up new avenues for therapeutic intervention of stress-related disorders. Just as CRH neurons and/or receptors are targets for prevention and therapy for depression and anxiety-related disorders, GRP and/or NMB neurons and/or their respective receptors may also be considered potential therapeutic targets.

1 Anisman H. and Merali Z. (2000) Understanding stress: characteristics and caveats. Alcohol Research and Health **23:** 241–249
2 Sapolsky R. M., Romero L. M. and Munck A. U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. Endocrine Reviews **21:** 55–89
3 Weninger S. C., Dunn A. J., Muglia L. J., Dikkes P., Miczek K. A., Swiergiel A. H. et al. (1999) Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. Proc. Natl. Acad. Sci. **96:** 8283–8288
4 Johnson E. O., Kamilaris T. C., Chrousos G. P. and Gold P. W. (1992) Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. Neurosci. Biobehav. Rev. **16:** 115–130
5 Kvetnansky R., Pacak K., Fukuhara K., Viskupic E., Hiremagalur B., Nankova B. et al. (1995) Sympathoadrenal system in stress. Ann. N.Y. Acad. Sci. **771:** 131–158
6 Michelson D., Licinio J. and Gold P. W. (1995) Mediation of the stress response by the hypothalamic-pituitary-adrenal axis. In: Neurobiological and Clinical Consequences of Stress, Freidman M. J. and. Charney D. S. (eds), pp. 225–238, Lippincott-Raven, Philadelphia
7 Bremner J. D., Krystal J. H., Southwick S. M. and Charney D. S. (1996) Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse **23:** 28–38
8 Herman J. P. and Cullinan W. E. (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. **20:** 78–84
9 Stratakis C. A. and Chrousos G. P. (1995) Neuroendocrinology and pathophysiology of the stress system. Ann. N.Y. Acad. Sci. **771:** 1–18
10 Morrow B. A., Redmond A. J., Roth R. H. and Elsworth J. D. (2000) The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. Brain Res. **864:** 146–151
11 Anastasi A., Erspamer V. and Bucci M. (1971) Isolation and structure of bombesin and alytesin, two analogous active peptides from the skin of the european amphibian bombina and alytes. Experientia **27:** 166–167
12 McDonald T. J., Jornvall H., Nilsson G., Vagne M., Ghatei M. A., Bloom S. R. and Mutt V. (1979) Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. Biochem. Biophys. Res. Commun. **90:** 227–237
13 Minamino N., Kangawa K. and Matsuo H. (1983) Neuromedin B: a novel bombesin-like peptide identified in porcine spinal cord. Biochem. Biophys. Res. Commun. **114(2)**, 541–548
14 Mantey S. A., Weber H. C., Sainz E., Akeson M., Ryan R. R., Pradhan T. K. et al. (1997) Discovery of a high affinity radioligand for the human orphan receptor, bombesin receptor subtype 3, which demonstrates that it has a unique pharmacology compared with other mammalian bombesin receptors. J. Biol. Chem. **272:** 26062–26071
15 Cullinan W. E., Herman J. P., Battaglia D. F., Akil H. and Watson S. J. (1995) Pattern and time course of immediate early

gene expression in rat brain following acute stress. Neuroscience **64:** 477–505
16 Senba E., Umemoto S., Kawai Y. and Noguchi K. (1994) Differential expression of fos family and jun family mRNAs in the rat hypothalamo-pituitary-adrenal axis after immobilization stress. Mol. Brain Res. **24:** 283–294
17 Honkaniemi J., Kononen J., Kainu T., Pyykonen I. and Pelto-Huikko M. (1994) Induction of multiple immediate early genes in rat hypothalamic paraventricular nucleus after stress. Brain Res. Mol. Brain Res. **25:** 234–241
18 Imaki T., Naruse M., Harada S., Chikada N., Nakajima K., Yoshimoto T. et al. (1998) Stress-induced changes of gene expression in the paraventricular nucleus are enhanced in spontaneously hypertensive rats. J. Neuroendocrinol. **10:** 635–643
19 Imaki T., Shibasaki T., Chikada N., Harada S., Naruse M. and Demura H. (1996) Different expression of immediate-early genes in the rat paraventricular nucleus induced by stress: relation to corticotropin-releasing factor gene transcription. Endocr. J. **43:** 629–638
20 Panula P., Yang H. Y. T. and Costa E. (1982) Neuronal location of the bombesin-like immunoreactivity in the central nervous system of the rat. Regul. Pept. **4:** 275–283
21 Giraud A. S., Smith A. I. and Rundle S. E. (1987) Distribution and molecular forms of immunoreactive bombesin in the ovine median eminence. Mol. Cell. Endocrinol. **53:** 245–250
22 Larsen P. J., O'Hare M. M. T., Vangsted A. and Mikkelsen J. D. (1989) Gastrin releasing peptide (GRP) is present in a GRP (1-27) form in anterior pituitary cells of the guinea pig. Peptides **10:** 815–818
23 Lemaire S., Chouinard L., Mercier P. and Day R. (1986) Bombesin-like immunoreactivity in bovine adrenal medulla. Regul. Pept. **13:** 133–146
24 Wada E., Way J., Lebacq-Verheyden A. M. and Battey J. F. (1990) Neuromedin B and gastrin-releasing peptide mRNAs are differentially distributed in the rat nervous system. J. Neurosci. **10:** 2917–2930
25 Battey J. and Wada E. (1991) Two distinct receptor subtypes for mammalian bombesin-like peptides. Trends Neurosci. **14:** 524–528
26 Houben H., Vandenbroucke A.-T., Verheyden A.-M. and Denef C. (1993) Expression of the genes encoding bombesin-related peptides and their receptors in anterior pituitary tissue. Mol. Cell. Endocrinol. **97:** 159–164
27 Moody T. W., Getz R., O'Donohue T. L. and Rosenstein J. M. (1988) Localization of receptors for bombesin-like peptides in the rat brain. Ann. N.Y. Acad. Sci. **547:** 114–130
28 Pert A., Moody T. W., Pert C. B., Dewald L. A. and Rivier J. (1980) Bombesin: receptor distribution in brain and effects on nociception and locomotor activity. Brain Res. **193:** 209–220
29 Zarbin M. A., Kuhar M. J., O'Donohue T. L., Wolf S. S. and Moody T. W. (1985) Autoradiographic localization of (125I-tyr4)bombesin-binding sites in rat brain. J. Neurosci. **5(2)**, 429–437
30 Wolf S. S., Moody T. W., O'Donohue T. L., Zarbin M. A. and Kuhar M. J. (1983) Autoradiographic visualization of rat brain binding sites for bombesin-like peptides. Eur. J. Pharmacol. **87:** 163–164
31 Ladenheim E. E., Jensen R. T., Mantey S. A. and Moran T. H. (1992) Distinct distributions of two bombesin receptor subtypes in the rat central nervous system. Brain Res. **593:** 168–178
32 Moody T. W., O'Donohue T. L. and Jacobowitz D. M. (1981) Biochemical localization and characterization of bombesin-like peptides in discrete regions of rat brain. Peptides **2:** 75–80
33 Chronwall B. M., Pisano J. J., Bishop J. F., Moody T. W. and O'Donohue T. L. (1985) Biochemical and histochemical characterization of ranatensin immunoreactive peptides in rat brain: lack of coexistence with bombesin/GRP. Brain Res. **338:** 97–113
34 Lee K., Dixon A. K., Gonzalez I., Stevens E. B., McNulty S., Oles R. et al. (1999) Bombesin-like peptides depolarize rat hippocampal interneurones through interaction with subtype 2 bombesin receptors. J. Physiol. **518:** 791–802
35 Kent P., Anisman H. and Merali Z. (1998) Are bombesin-like peptides involved in the mediation of stress response? Life Sci. **62:** 103–114
36 Merali Z., Kent P., Michaud D., McIntyre D. and Anisman H. (2001) Differential effects of psychogenic and neurogenic stressors on central corticotropin-releasing hormone and bombesin-like peptides in Fast and Slow seizing rat. Brain Res.
37 Chan R. K., Brown E. R., Ericsson A., Kovacs K. J. and Sawchenko P. E. (1993) A comparison of two immediate-early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry. J. Neurosci. **13:** 5126–5138
38 Palkovits M., Baffi J. S. and Dvori S. (1995) Neuronal organization of stress response: pain-induced c-fos expression in brainstem catecholaminergic cell groups. Ann. N.Y. Acad. Sci. **771:** 313–326
39 Merali Z., McIntosh J., Kent P., Michaud D. and Anisman H. (1998) Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. J. Neurosci. **18:** 4758–4766
40 Kent P., Anisman H. and Merali Z. (2001) Central bombesin activates the hypothalamic-pituitary-adrenal axis: effects on regional levels and release of corticotropin-releasing hormone and arginine vasopressin. Neuroendocrinology **73:** 203–214
41 Au C. L., Canny B. J., Farnworth P. G. and Giraud A. S. (1997) Bombesin regulation of adrenocorticotropin release from ovine anterior pituitary cells. Peptides **18:** 995–1000
42 Garrido M. M., Manzanares J. and Fuentes J. A. (1999) Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of adrenocorticotropin and corticosterone secretion by gastrin-releasing peptide. Brain Res. **828:** 20–26
43 Garrido M. M., Martin S., Ambrosio E., Fuentes J. A. and Manzanares J. (1998) Role of corticotropin-releasing hormone in gastrin-releasing peptide-mediated regulation of corticotropin and corticosterone secretion in male rats. Neuroendocrinology **68:** 116–122
44 Olsen L., Knigge U. and Warberg J. (1992) Gastrin-releasing peptide stimulation of corticotropin secretion in male rats. Endocrinology **130:** 2710–2716
45 Gunion M. W., Tache Y., Rosenthal M. J., Miller S., Butler B. and Zib B. (1989) Bombesin microinfusion into the rat hypothalamic paraventricular nucleus increases blood glucose, free fatty acids and corticosterone. Brain Res. **478:** 47–58
46 Malendowicz L. K. and Nussdorfer G. G. (1995) Investigations on the acute effects of neuropeptides on the pituitary-adrenocortical function in normal and cold- stressed rats. I. Bombesin and neuromedin B. Exp.Toxicol. Pathol. **47:** 31–34
47 Malendowicz L. K. (1998) Role of neuromedins in the regulation of adrenocortical function. Hormone and Metabolic Research **30:** 374–383
48 Sander L. D. and Porter J. R. (1988) Influence of bombesin, CCK, secretin and CRF on corticosterone concentration in the rat. Peptides **9:** 113–117
49 Sander L. D. and Thomas R. M. (1991) Gastrin releasing peptide, but not pentagastrin, stimulates ACTH and cortisol secretion in conscious dogs. Regul. Pept. **35:** 127–133
50 Thomas R. M. and Sander L. D. (1985) Influence of CCK and bombesin on ACTH and cortisol secretion in the conscious dog. Peptides **6:** 703–707
51 Knigge U., Holst J. J., Knuhtsen S., Bach F. W. and Bang P. (1987) Corticotropin-releasing activity of gastrin-releasing peptide in normal men. J. Clin. Endocrinol. Metab. **65:** 1291–1295

52 Brown M., Tache Y. and Fisher D. (1979) Central nervous system action of bombesin: mechanism to induce hyperglycemia. Endocrinology **105:** 660–665
53 Brown M. R., Carver K. and Fisher L. A. (1988) Bombesin: central nervous system actions to affect the autonomic nervous system. Ann. N.Y. Acad. Sci. **547:** 174–182
54 Carver-Moore K., Gray T. S. and Brown M. R. (1991) Central nervous system site of action of bombesin to elevate plasma concentrations of catecholamines. Brain Res. **541:** 225–231
55 Okuma Y., Yokotani K. and Osumi Y. (1996) Brain prostaglandins mediate the bombesin-induced increase in plasma levels of catecholamines. Life Sci. **59:** 1217–1225
56 Plamondon H. and Merali Z. (1993) Effects of central neuromedin B and related peptides on blood glucose. Regul. Pept. **47:** 133–140
57 Iguchi A., Matsunaga H., Nomura T., Gotoh M. and Sakamoto N. (1984) Glucoregulatory effects of intrahypothalamic injections of bombesin and other peptides. Endocrinology **114:** 2242–2246
58 Okuma Y., Yokotani K. and Osumi Y. (1991) Chemical sympathectomy with 6-hydroxydopamine potentiates intracerebroventricularly applied bombesin-induced increase in plasma adrenaline. Life Sci. **49:** 1611–1618
59 Okuma Y., Yokotani K. and Osumi Y. (1995) Centrally applied bombesin increases nerve activity of both sympathetic and adrenal branch of the splanchnic nerves. Jpn. J. Pharmacol. **68:** 227–230
60 Fisher L. A., Cave C. R. and Brown M. R. (1985) Central nervous system cardiovascular effects of bombesin in conscious rats. Am. J. Physiol. **248:** H425–H431
61 Kulkosky P. J., Gibbs J. and Smith G. P. (1982) Behavioral effects of bombesin administration in rats. Physiol. Behav. **28:** 505–512
62 Muurahainen N. E., Kissileff H. R. and Pi-Sunyer F. X. (1993) Intravenous infusion of bombesin reduces food intake in humans. Am. J. Physiol. **264:** R350–R354
63 Johnston S. A. and Merali Z. (1988) Specific neuroanatomical and neurochemical correlates of locomotor and grooming effects of bombesin. Peptides **9 (Suppl. 1):** 245–256
64 Itoh S., Takashima A., Itoh T. and Morimoto T. (1994) Open-field behavior of rats following intracerebroventricular administration of neuromedin B, neuromedin C and related amphibian peptides. Jpn. J. Physiol. **44:** 271–281
65 Dunn A. J. and Berridge C. W. (1990) Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses. Brain Res. Rev. **15:** 71–100
66 Krahn D. D., Gosnell B. A., Grace M. and Levine A. S. (1986) CRF antagonist partially reverses CRF- and stress-induced effects on feeding. Br. Res. Bull. **17:** 285–289
67 Denbow D. M. (1989) Centrally and peripherally administered bombesin decreases food intake in turkeys. Peptides **10:** 275–279
68 Taylor I. L. and Garcia R. (1985) Effects of pancreatic polypeptide, caerulein, and bombesin on satiety in obese mice. Am. J. Physiol. **248:** 6277–6280
69 Gibbs J., Fauser D. J., Rowe E. A., Rolls B. J., Rolls E. T. and Maddison S. P. (1979) Bombesin suppresses feeding in rats. Nature **282:** 208–210
70 Plamondon H. and Merali Z. (1997) Anorectic action of bombesin requires receptor for corticotropin-releasing factor but not for oxytocin. Eur. J. Pharmacol. **340:** 99–109
71 Li B.-H. and Rowland N. E. (1996) Peripherally and centrally administered bombesin induce Fos-like immunoreactivity in different brain regions in rats. Regul. Pepti. **62:** 167–172
72 Plamondon H. and Merali Z. (1997) Regulation of ingestion by CRF and bombesin-like peptides: distinct meal-related peptide level changes. Am. J. Physiol. **272:** R268–R274
73 Plamondon H. and Merali Z. (1994) Push-pull perfusion reveals meal-dependent changes in the release of bombesin-like peptides in the rat paraventricular nucleus. Brain Res. **668:** 54–61
74 Ladenheim E. E. and Ritter R. C. (1993) Caudal hindbrain participation in the suppression of feeding by central and peripheral bombesin. Am. J. Physiol. **264:** R1229–R1234
75 LaCour F. J., Knott J. N., Kenney N. J. and Woods S. C. (1986) Intraventricular bombesin (BBS) reduces food intake of normal but not area-postrema-lesioned rats. Appetite **7:** 273–274
76 Ferre P., Fernandez-Teruel A., Escorihuela R. M., Driscoll P., Corda M. G., Giorgi O. et al. (1995) Behavior of the Roman/Verh high- and low-avoidance rat lines in anxiety tests: relationship with defecation and self-grooming. Physiol. Behav. **58:** 1209–1213
77 Abraham M. E. and Gogate M. G. (1989) Effect of stress on behaviour in rats. Ind. J. Physiol. Pharmacol. **33:** 84–88
78 Spruijt B. M., Hooff J. A. R. A. M. van and Gispen W. H. (1992) Ethology and neurobiology of grooming behavior. Physiol. Rev. **72:** 825–852
79 Gmerek D. E. and Cowan A. (1983) Studies on bombesin-induced grooming in rats. Peptides **4:** 907–913
80 Cowan A. (1988) Behavioral effects of bombesin. Ann. N.Y. Acad. Sci. **547:** 204–209
81 Merali Z. and Piggins H. (1990) Effects of dopamine D_1 and D_2 receptor agonists and antagonists on bombesin-induced behaviors. Eur. J. Pharmacol. **191:** 281–293
82 Merali Z., Kateb C. and Kateb P. (1988) Role of the cholinergic system(s) in the expression of the behavioral effects of bombesin. Ann. N.Y. Acad. Sci. **525:** 423–424
83 Van Wimersma G., van de Brug F., de Bruijckere L. M., Pabst P. H., Ruesink R. W., Hulshof R. L. et al. (1988) Comparison of bombesin-, ACTH- and beta-endorphin-induced grooming. Antagonism by haloperidol, naloxone and neurotensin. Ann. N.Y. Acad. Sci. **525:** 219–227
84 Merali Z., Johnston S. and Zalcman S. (1983) Bombesin-induced behavioral changes: antagonism by neuroleptics. Peptides **4:** 693–697
85 Schulz D. W., Kalivas P. W., Nemeroff C. B. and Prange A. J. Jr (1984) Bombesin-induced locomotor hyperactivity:evaluation of the involvement of the mesolimbic dopamine system. Brain Res. **304:** 377–382
86 Bodnoff S. R., Suranyi-Cadotte B., Quirion R. and Meaney M. J. (1987) Postnatal handling reduces novelty-induced fear and increases (3H)flunitrazepam binding in rat brain. Eur. J. Pharmacol. **144:** 105–107
87 Muller-Gass A., Porath A., Anisman H. and Merali Z. (2000) Novelty-induced delay in snack approach: a robust and rapid naturalistic model for anxiety assessment. Soc. Neurosci. Abstr. **30:** 659.2
88 Ashwood V., Brownhill V., Higginbottom M., Horwell D. C., Hughes J., Lewthwaite R. A. et al. (1998) PD 176252 – the first high affinity non-peptide gastrin-releasing peptide (BB2) receptor antagonist. Bioorg. Medic. Chem. Lett. **8:** 2589–2594
89 Ryan R. R., Mantey S. A., Pradhan T. K., Battey J. F. and Jensen R. T. (1999) Comparative pharmacology of PD 108368, a non-peptide neuromedin B antagonist. FASEB J. **13:** A466
90 Lewis J. W., Cannon T. J and Liebeskind J. C. (1980) Opioid and nonopioid mechanisms of stress analgesia. Science **208:** 623–625
91 Lewis J. W., Sherman J. E. and Liebeskind J. C. (2001) Opioid and non-opioid stress analgesia: assessment of tolerance and cross-tolerance with morphine. J. Neurosci. **1:** 358–363
92 Kent P., Bedard T., Kahn S. E., Anisman H. and Merali Z. (2001) Bombesin-induced HPA and sympathetic activation requires CRH receptors. Peptides **22:** 57–65
93 Familari M., Funder J. W. and Giraud A. S. (1988) Potentiation by bombesin of corticotropin-releasing factor- stimulated

ACTH release is dependent on the presence of glucocorticoids. Ann. N.Y. Acad. Sci. **547:** 505–507
94 Kent P., Anisman H. and Merali Z. (2001) Central bombesin administration elicits site-specific alterations in the levels of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) immunoreactivity. Brain Res., in press
95 Strand F. L. (1999) Neuropeptides: Regulators of Physiological Processes. MIT Press, Cambridge, MA
96 Cullinan W. E., Herman J. P., Heimrich D. L., Stanley W. J. (1995) A neuroanatomy of stress. In: Neurobiological and Clinical Consequences of Stress: From Normal Adaptation to Post-traumatic Stress Disorder, pp. 3-26, Freidman M. J. and Charney D. S. (eds), Lippincott-Raven, Philadelphia
97 Cunningham E. T., Sawchenko P. E. (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J. Comp. Neurol. **274:** 60–76
98 Cunningham E. T. J., Bohn M. C. and Sawchenko P. E. (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J. Compa. Neurol. **292:** 651–667
99 Lynn R. B., Hyde T. M., Cooperman R. R. and Miselis R. R. (1996) Distribution of bombesin-like immunoreactivity in the nucleus of the solitary tract and dorsal motor nucleus of the rat and human: colocalization with tyrosine hydroxylase. J. Comp. Neurol. **369:** 552–570
100 Costello J. F., Brown M. R. and Gray T. S. (1991) Bombesin immunoreactive neurons in the hypothalamic paraventricular nucleus innervate the dorsal vagal complex in the rat. Brain Res. **542:** 77–82
101 Whitnall M. H., Mezey E. and Gainer H. (1985) Co-localzation of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. Nature **317:** 248–250
102 Cooper S. J. and Dourish C. T. (1990) Multiple cholecystokinin (CCK) receptors and CCK-monoamine interactions are instrumental in the control of feeding. Physiol. Behav. **48:** 849–857
103 Gold P. W., Kaye W., Robertson G. L. and Ebert M. (1983) Abnormalities in plasma and cerebrospinal-fluid arginine vasopressin in patients with anorexia nervosa. N. Engl. J. Med. **308:** 1117–1123
104 Plotsky P. M., Owens M. J. and Nemeroff C. B. (1998) Psychoneuroendocrinology of depression. Psychoneuroendocrinology , 293–307
105 Arato M., Banki C. M., Bissette G. and Nemeroff C. B. (1989) Elevated CSF CRF in suicide victims. Biol. Psychiatr. **25:** 355–359
106 Nemeroff C. B., Widerlov E., Bissette G., Walleus H., Karlsson, Eklund K. et al. (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science **226:** 1342–1344
107 Banki C. M., Bissette G., Arato M., O'C and Nemeroff C. B. (1987) CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. Am. J. Psychiatr. **144:** 873–877
108 Raadsheer F. C., Hoogendijk W. J., Stam F. C., Tilders F. J. and Swaab D F. (1994) Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology **60:** 436–444
109 Nemeroff C. B., Owens M. J., Bissette G., Andorn A. C. and Stanley M. (1988) Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. Arch. Gen. Psychiatr. **45:** 577–579
110 Purba J. S., Hoogendijk W. J., Hofman M. A. and Swaab D. F. (1996) Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. Arch. Gen. Psychiatr. **53:** 137–143