Published in final edited form as:

Cell Mol Neurobiol. 2018 January ; 38(1): 25–35. doi:10.1007/s10571-017-0543-8.

Regulation of Hypothalamo-Pituitary-Adrenocortical Responses to Stressors by the Nucleus of the Solitary Tract/Dorsal Vagal Complex

James P. Herman¹

¹Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati, Cincinnati OH, 45237

Abstract

Hindbrain neurons in the nucleus of the solitary tract are critical for regulation of the hypothalamo-pituitary-adrenocortical (HPA) responses to stress. It is well-known that noradrenergic (as well as adrenergic) neurons in the NTS send direct projections to hypophysiotrophic corticotropin-releasing hormone (CRH) neurons and control activation of HPA axis responses to acute systemic (but not psychogenic) stressors. Norepinephrine (NE) signaling via α_1 receptors is primarily excitatory, working either directly on CRH neurons or through presynaptic activation of glutamate release. However, there is also evidence for NE inhibition of CRH neurons (possibly via β receptors), an effect that may occur at higher levels of stimulation, suggesting that NE effects on the HPA axis may be context-dependent. Lesions of ascending NE inputs to the PVN attenuate stress-induced ACTH but not corticosterone release after chronic stress, indicating reduction in central HPA drive and increased adrenal sensitivity. Non-catecholaminergic NTS glucagon-like peptide 1 (GLP-1)/glutamate neurons play a broader role in stress regulation, being important in HPA activation to both systemic and psychogenic stressors as well as HPA axis sensitization under conditions of chronic stress. Overall, the data highlight the importance of the NTS as a key regulatory node for coordination of acute and chronic stress.

Keywords

corticotropin-releasing hormone; glucagon-like peptide-1; glucocorticoids; norepinephrine; paraventricular nucleus; prolactin releasing peptide

It is a pleasure to contribute an article for this special issue honoring Richard Kvetnansky's life. I first became acquainted with Richard as many of us did, as a participant in the series of International Symposia on Catecholamines and Other Neurotransmitters in Stress (Jezova and Herman 2016). I was first invited to the symposium in 1999. In keeping with the primary emphasis of the conference, for my first presentation Richard asked me to focus my talk on the role of catecholamine circuitry in regulation of stress processes. Thus, it is only appropriate that the current contribution reviews the role of the nucleus of the solitary tract/

dorsal vagal complex, a cell group that prominently features catecholaminergic neurons, on hypothalamo-pituitary-adrenocortical (HPA) axis responses to acute and chronic stress.

Neuroendocrine stress activation is controlled by corticotrophin-releasing hormone (CRH) neurons in the medial parvocellular division of the hypothalamic paraventricular nucleus (PVN). In addition to CRH, these neurons also produce numerous other neuropeptides, the most important of which is arginine vasopressin (AVP). Axons of CRH neurons form neurohemal contacts on blood vessels in the external lamina of the median eminence, affording access of released peptides to promote ACTH release by corticotropes in the anterior pituitary. Release of ACTH requires stimulation by CRH, but is greatly augmented by co-release of AVP. ACTH then travels via the systemic circulation to the adrenal cortex, where it promotes synthesis and release of glucocorticoids (corticosterone in rats and mice). Glucocorticoids have widespread actions on nearly all organ systems, causing a general mobilization of energy resources and altering transcription of a wide variety of genes. Glucocorticoids released during times of stress or demand act via multiple mechanisms to exert negative feedback inhibition on PVN neurons, preventing excessive secretion (Herman et al. 2016).

It is well known that the HPA axis is subject to regulation by the hindbrain. Early studies note that posterior deafferentation of the hypothalamus reduces the ability of stressors to activate the HPA axis (e.g., (Siegel et al. 1980)). Subsequent work has supported a largely excitatory role of brainstem nuclei on ACTH and corticosterone release, attributed to ascending information signaling systemic dyshomeostasis (e.g., blood loss, respiratory distress, etc) (Herman and Cullinan 1997; Sawchenko et al. 2000) and more recently, possible relay of descending information from limbic forebrain structures (Ulrich-Lai and Herman 2009). Much of the work has identified the NTS as a primary mediator of many of these HPA axis excitatory responses. This review will summarize evidence for involvement of NTS inputs, including those from catecholaminergic neurons, in drive of PVN CRH neurons and HPA axis responses by stressors.

Regulation of the HPA Axis by the NTS: Catecholamines

Anatomical evidence suggests direct actions of A2 neurons on CRH neurons in the PVN. Catecholaminergic inputs onto the CRH-containing region of the PVN emanate primarily from cell bodies in the A2 (and C2) region of the NTS/dorsal vagal complex (heretofore designated as the NTS) (Cunningham et al. 1990; Cunningham and Sawchenko 1988). Axons of noradrenergic and adrenergic neurons ascend in the ventral noradrenergic bundle (as part of the visceral lemniscus). It is important to note that both the A1 region and the locus coeruleus (LC) also contribute innervation to the PVN, with the former largely innervating the magnocellular divisions and the latter, the more medial periventricular region (Cunningham et al. 1990; Cunningham and Sawchenko 1988). Recent evidence suggests that these cell groups also provide some innervation to the medial parvocellular zone, albeit less than that of the A2 region. The medial parvocellular zone receives the highest concentration of NE afferents of all PVN subdivisions, and also receives substantial adrenergic input (Cunningham et al. 1990; Cunningham and Sawchenko 1988). Both light and electron microscopic analysis provide evidence of NE/E containing terminals and synaptic

specializations onto the cell bodies of PVN CRH neurons (Liposits et al. 1986a; Liposits et al. 1986b), verifying the capacity for direct interaction with CRH neurons. Medial parvocellular PVN neurons express alpha(1), alpha(2), beta(1) and beta(2) receptor subunits (Nicholas et al. 1993; Pieribone et al. 1994; Day et al. 1999; Sands and Morilak 1999), and provide a potential substrate for local NE action through multiple mechanisms.

Microdialysis studies confirm that NE is released in the PVN following exposure to a variety of acute stressors (Pacak et al. 1995a; Pacak et al. 1995b), indicating a role in modulating activity of the HPA axis. The amount of NE release is related to the magnitude of ACTH release, as determined from comparison with the area under the curve of the hormone response (Pacak et al. 1995b). Local infusion of NE or alpha (1)-adrenergic agonists causes ACTH and corticosterone secretion as well as release of CRH into the portal circulation (Plotsky 1987; Szafarczyk et al. 1987). Conversely, alpha (1)-adrenergic antagonists block HPA axis responses to cytokine infusion (Plotsky 1987), confirming the physiologic importance of medial parvocellular NE release. Local NE also promotes PVN Fos mRNA expression and CRH and AVP gene transcription (as well as corticosterone release) (Cole and Sawchenko 2002; Itoi and Sugimoto 2010), consistent with NE being a trigger for stimulus: transcription coupling. Finally, in vivo electrophysiological studies indicate that low frequency stimulation of ascending NE axons in the ventral noradrenergic bundle (VNAB) excites median eminence-projecting neurons in an alpha(1) dependent fashion (Saphier and Feldman 1991). Collectively, these data are consistent with a role for NE in stress excitation, mediated by alpha (1) adrenergic receptors.

However, other lines of evidence suggest that the role of NE (and perhaps E) in the PVN may be complex. Whereas low frequency VNAB stimulation is excitatory, high frequency stimuli inhibits median eminence projecting neurons via beta adrenergic receptors (Saphier and Feldman 1991). Moreover, NE can inhibit a subset of parvocellular neurons by a beta-receptor mediated mechanism (Daftary et al. 2000). Similarly, NE inhibits a subpopulation of parvocellular PVN neurons in slice preparations, an effect that again is blocked by beta (but not alpha) receptor antagonism (Han et al. 2002). Finally, central infusion of a high dose of NE can reduce release of CRH into the portal circulation, which is reversible by alpha(2) or beta receptor antagonists (Plotsky 1987). Collectively, these studies suggest the impact of NE neurons may depend on the degree of NTS neuron activation and NE release, with PVN effects mediated through different receptor subtypes.

There is also evidence for local interactions of NE with glutamatergic or GABAergic neurons in the region of the PVN. In slice preparations, NE increases ePSC frequency in parvocellular PVN neurons. The excitatory effect of NE was blocked by prazosin but not propranolol or timolol, indicating mediation by alpha (1) receptors. However, the excitatory effect could be blocked by ionotropic glutamate receptor antagonists as well as tetrodotoxin, indicating that NE effects are mediated by local release of glutamate rather than (or in addition to) directly at the level of NE synapses (Daftary et al. 1998). Notably, some 80% of NTS TH neurons express glutamate transporter 2 (Stornetta et al. 2002), suggesting the possibility of glutamate and NE co-release in ascending PVN afferents. NE can either stimulate or inhibit GABAergic spontaneous inhibitory synaptic currents in parvocellular neurons, via postsynaptic alpha (1) receptors or presynaptic alpha (2) receptors, respectively

(Han et al. 2002). In addition, NE is responsible for stress-induced metaplasticity at GABAergic synapses, mediated via beta receptors (Inoue et al. 2013). The latter studies suggest that adrenergic receptors may also play a role in feedback inhibition of CRH neurons, perhaps via modulation of GABAergic inputs.

Lesions compromising NTS afferents to the PVN have modality-specific effects on HPA axis responses to stress. Lesions of the VNAB with 6-OHDA, a catecholaminergic neurotoxin, reduce CORT responses to ether stress and (to a lesser extent) insulin (Gaillet et al. 1993; Szafarczyk et al. 1987; Herman et al. 2002), but not to restraint or histamine (Gaillet et al. 1993; Szafarczyk et al. 1987; Herman et al. 2002). Lesions of the VNAB compromise ascending afferents from the A2/C2 (NTS) and A1/C1 regions (but not locus coeruleus), and cause extensive depletion of hypothalamic NE (Gaillet et al. 1993; Szafarczyk et al. 1987). Reduced HPA responses to ether were larger when placed in the dorsal VNAB, which contains afferents from the NTS region (Gaillet et al. 1993). Subsequent work from our group corroborated the effects of VNAB lesion on corticosterone responses to ether stress, but not on responses to restraint (Herman et al. 2002). Lesions of the VNAB also decrease ACTH release and induction of PVN CRH hnRNA (a measure of CRH transcription) following foot shock stress (Blandino et al. 2013). However, corticosterone secretion was not reduced in concert with ACTH (Blandino et al. 2013). It is important to also note that prior studies using unilateral lesion of ascending catecholaminergic pathways reduce PVN Fos activation and CRH mRNA expression following ul-1b but not footshock (Li et al. 1996), suggesting that the necessity of these pathways for PVN activation may be differentially lateralized. VNAB lesions also result in depletion of PVN and median eminence CRH peptide (Alonso et al. 1986), consistent with reduced response capacity of the HPA axis.

More directed approaches largely agree with the results seen following large VNAB denervation. Ibotenic acid lesions of the NTS reduce Fos expression in PVN CRH (as well as oxytocin) neurons following IL-1b (Buller et al. 2001). Selective deafferentation of the PVN by intranuclear injection of DBH-conjugated saporin resulted in attenuated CORT release following insulin or 2-DG infusion, but not following swim stress (Ritter et al. 2003). Induction of CRH gene transcription (CRH hnRNA expression) by 2-DG was also reduced (Ritter et al. 2003), consistent with decreased central activation of the HPA axis. Similarly, DSAP injections into the PVN attenuate corticosterone responses, CRH mRNA induction and Fos activation in response to immune stimulation (lipopolysaccharide or IL-1b injection) but not restraint (Schiltz and Sawchenko 2007; Bienkowski and Rinaman 2008), again indicative of stressor-specific modulation of PVN activation by NE.

The effects of VNAB lesions differentially affect ACTH vs corticosterone responses to restraint (Gaillet et al. 1993) and footshock (Blandino et al. 2013), generally thought of as stressors of psychogenic origin. In both cases, a normal corticosterone response is evident, despite substantial reduction of ACTH. In combination with the marked reduction in CRH peptide, the data suggest that lesions of PVN projecting hindbrain NE/E neurons reduce central activation of the HPA axis following chronic stress, but enhance the sensitivity of the adrenal to ACTH (Blandino et al. 2013; Gaillet et al. 1993). Altered adrenal sensitivity is a common phenomenon, seen in numerous conditions and situations, including chronic stress

exposure, systemic inflammation, aging and a variety of central stress circuit manipulations (Bornstein et al. 2008; Spiga and Lightman 2015; Ulrich-Lai et al. 2006b).

The mechanism of the ACTH:corticosterone dissociation following VNAB lesion has yet to be defined. It is possible that the NTS modulates adrenal sensitivity through actions on the sympathetic nervous system. Indeed, recent work indicates that DSAP lesions of the NTS increase blood pressure responses to acute stress (Daubert et al. 2012). Notably, both DSAP and 6-OHDA lesions of the NTS decrease peak corticosterone release following restraint (Bundzikova-Osacka et al. 2015; Daubert et al. 2012), indicating that NE neurons in this region differentially regulate autonomic vs. neuroendocrine responses. It remains to be determined whether NTS lesions directly enhance drive of the adrenal by the sympathetic nervous system or increase adrenal blood flow (and hence availability of ACTH).

It is possible that differences in HPA axis and autonomic responses to stress may be mediated by glucocorticoids. Work from our group demonstrates that corticosterone implants into the NTS region reduce stress-induced ACTH and corticosterone secretion, whereas, implants of mifepristone enhance HPA stress reactivity (Ghosal et al. 2014). In contrast, corticosterone implants into the NTS increase blood pressure responses to stress and elevate basal circulating NE and neuropeptide Y, while decreasing peak CORT responses to restraint (Daubert et al. 2014; Scheuer 2010). It is thought that enhanced pressor responses to stress are mediated by enhanced activation of magnocellular vasopressin neurons in the PVN and supraoptic nuclei (Daubert et al. 2014).

Physiological conditions that reduce HPA axis activity are frequently associated with withdrawal of NE action at the PVN. For example, pregnancy markedly reduces HPA axis activation to a variety of stressful stimuli, e.g., inflammation (IL-1 beta injection). Loss of HPA excitability is linked to inhibition of hindbrain NE neurons by local opioid peptide mechanisms (Russell et al. 2008). Similarly, HPA axis hyporesponsiveness observed during lactation is linked to reduced PVN NE release (Toufexis et al. 1998). In rats, long-term self-administration of nicotine reduces ACTH secretion and PVN NE release to mild footshock, in parallel with reduced activation of NE neurons in the A2 and C2 regions (Yu and Sharp 2010). Notably, in the latter study ACTH secretion by pharmacological activation of alpha (1)-receptors was potentiated, suggesting an adaptive response to loss of NE drive (Yu and Sharp 2010).

The role of NTS catecholamines in acute stress regulation suggests that this circuitry is also important in chronic stress-induced HPA axis hypersecretion. In keeping with this possibility, exposure to chronic stress (chronic variable stress (CVS) or repeated restraint) increases expression of tyrosine hydroxylase mRNA expression and gene transcription in the NTS (Toth et al. 2008; Zhang et al. 2010). Increased TH mRNA expression is accompanied by increases in TH immunoreactivity in the PVN (Zhang et al. 2010). Importantly, TH up-regulation is not observed in the locus coeruleus (Herman et al. 2002), implying that CVS selectively drives TH synthesis in the hindbrain. Increased expression of LC TH mRNA can be observed following isolation or social subordination (Watanabe et al. 1995), possibly due to differential engagement by chronic social stress. However, decreases in LC TH mRNA are observed following CVS or chronic mild stress (Duncko et al. 2001; Herman et al. 2002),

indicating that stress engagement of the LC may be modality specific. Importantly, exposure to CVS also increases the numbers of dopamine beta hydroxylase-positive appositions onto CRH neurons in the medial parvocellular PVN (Flak et al. 2009), which is preferentially innervated by the NTS. Collectively, these data suggest increased NTS NE bioavailability following chronic stress.

However, despite evidence for increased NE synthesis and PVN innervation, the data to date are not overwhelmingly consistent with a prominent role in chronic stress-induced HPA drive. There are some data to suggest that NE mediates sensitization of HPA axis responses by chronic cold, as blockade of alpha (1)-adrenergic receptors blocks potentiated ACTH release (Ma and Morilak 2005). Indeed, chronic cold potentiated the stimulatory effect of intra-PVN alpha (1)-agonist injections on ACTH (Ma and Morilak 2005), consistent with the observed increase in alpha(1)-sensitivity observed with chronic nicotine infusions (Yu and Sharp 2010). However, neither DSAP injections in the PVN nor 6-OHDA infusion in NTS significantly impact long-term markers of chronic stress, such as reduced weight gain and adrenal hypertrophy (Bundzikova-Osacka et al. 2015; Flak et al. 2014). In addition, lesions did not affect the corticosterone response to stress after CVS exposure. (Bundzikova-Osacka et al. 2015; Flak et al. 2014). However, it is important to note that ACTH responses are decreased following CVS in PVN DSAP-lesion rats (Flak et al. 2014). These data suggest that the adrenal compensates for enhanced central HPA activation, a possibility that is supported by increased adrenal sensitivity (as assessed by the corticosterone/logACTH ratio) (Engeland et al. 1981). Adrenal sensitivity is regulated by postganglionic sympathetic neurons that directly innervate the adrenal cortex (Buijs et al. 1999). Sympathetic splanchnic nerves promote blood flow to the adrenal (Engeland et al. 1985) and adrenal steroidogenesis (Ulrich-Lai et al. 2006a), increasing both delivery of ACTH and glucocorticoid synthetic capacity. Modulation of sympathetic drive may be mediated by targets of NTS neurons, including preautonomic neurons in the PVN (Buijs et al. 1999). It is important in this regard that 6-OHDA lesions of the NTS reduce CVS-induced increases in sympathetic tone to the heart, as reflected in an increase in the low frequency:high frequency ratio component of heart rate variability (Bundzikova-Osacka et al. 2015). Thus, it is possible that the NE innervation of the PVN is more important in driving autonomic consequences of chronic stress, which may account for the observed changes in adrenal sensitivity.

Regulation of the HPA Axis by the NTS: Neuropeptides

The NTS contains substantial populations of neuropeptidergic neurons. Some neuropeptides are known to co-localize with catecholaminergic markers (e.g., prolactin releasing peptide (PrRP)) (Maniscalco and Rinaman 2017). In addition, non-catecholaminergic GLP-1 neurons of the NTS also project to the medial parvocellular PVN (Larsen et al. 1997), suggesting the capacity for regulation of the HPA axis by multiple sources of NTS input. Generally, neuropeptides are released at relatively high stimulus intensity and often complement or oppose the actions of co-localized classical transmitters, such as NE, glutamate and GABA.

Glucagon-like peptide1 (GLP-1) is perhaps the best studied neuropeptide system in the NTS, largely because of its link to energy balance and malaise (Ghosal et al. 2013). The NTS and

neighboring lateral medulla contain the vast majority of GLP-1 neurons in brain (Larsen et al. 1997; Llewellyn-Smith et al. 2013; Merchenthaler et al. 1999), suggesting that physiologic actions of GLP-1 originate from one or both of these sources. Importantly, anatomical studies indicate that GLP-1 does not co-localize with catecholamines (Larsen et al. 1997). The majority of GLP-1 neurons also express vesicular glutamate transporter 2, indicating that they are glutamatergic (Zheng et al. 2015). The PVN is densely innervated by GLP-1 positive fibers (Sarkar et al. 2003; Tauchi et al. 2008b), which form synaptic appositions onto hypophysiotrophic CRH neurons in the medial parvocellular subnucleus (Sarkar et al. 2003). Importantly, the canonical GLP-1 receptor (Glp1r) is expressed in the medial parvocellular PVN (Merchenthaler et al. 1999) and co-localizes with CRH (Ghosal et al. 2017), consistent with the capacity for HPA axis regulation.

Physiological studies indicate that GLP-1 excites the HPA axis. Central (icv) administration of GLP-1 causes release of ACTH and corticosterone, whereas infusion of GLP1r antagonists (either icv or directly into the PVN) attenuate HPA axis responses to stress (Kinzig et al. 2003). Recent data from our group demonstrates that conditional deletion of Glp1r in PVN (using a Sim1 promoter) reduces HPA axis responses to restraint and hypoxia, and attenuates Fos activation of medial parvocellular PVN neurons (Ghosal et al. 2017). Interestingly, Glp1r antagonists and PVN deletion reduce HPA axis activation by both psychogenic (elevated platform, restraint) and systemic (LiCl, hypoxia) stressors (Kinzig et al. 2003, Ghosal et al. 2017), indicating that unlike catecholamines, GLP-1 mediates responses to a wide variety of stressors of varying intensity.

Also in contrast to the catecholamines, GLP-1 neurons appear to regulate HPA responsiveness to chronic stress. Daily central (icv) administration of the Glp1r antagonist des-His₁, glu₉-exendin 4 prior to stressor exposures blocks CVS-induced weight loss and sensitization of CORT responses to restraint, whereas daily GLP-1 infusions increase response magnitude and weight loss (Tauchi et al. 2008a). Involvement of PVN Glp1r in CVS-induced weight loss and CORT secretion was confirmed using mice with conditional deletion of Glp1r driven by the Sim1 promoter (Ghosal et al. 2017).

Our studies further suggest that GLP-1 neurons may be a target for glucocorticoid feedback regulation of the HPA axis. Expression of the gene encoding the GLP-1 precursor preproglucagon (PPG) in NTS is rapidly decreased by either acute stress or exogenous corticosterone administration. In addition, down-regulation of PPG mRNA is blocked in adrenalectomized, corticosterone replaced animals, indicative of regulation by stress-related corticosterone fluxes (Zhang et al. 2009). Expression of PPG hnRNA was increased following stress, indicating that down-regulation is likely post-transcriptional, perhaps at the level of RNA stability. Loss of transcript was accompanied by decreases in immunoreactive GLP-1 terminals in the PVN, consistent with local release (Zhang et al. 2009). Chronic stress exposure causes a similar, corticosterone-dependent loss in both NTS PPG mRNA and PVN GLP-1, indicating a possible compensatory down-regulation of this system following chronic stimulation (Zhang et al. 2010). Together, the data suggest that glucocorticoids act to down-regulate NTS PPG expression and GLP-1 release in the context of stress.

Like GLP-1 neurons, prolactin releasing peptide (PrRP) neurons are richly localized in the NTS (Maniscalco et al. 2012; Morales et al. 2000). Unlike GLP-1, PrRP is largely expressed in catecholaminergic neurons (>80% of PrRP neurons also contain TH) (Maruyama et al. 2001). There are data suggestive of immunopositive PrRP terminals appositions onto CRH neurons in the PVN (Matsumoto et al. 2000), and tracing studies indicating that PVN-projecting PrRP neurons originate in the NTS (and A1 region) (Morales et al. 2000; Uchida et al. 2010). Hindbrain PrRP neurons show Fos colocalization following exposure to multiple stressful stimuli (Maniscalco et al. 2015; Maruyama et al. 2001; Seal et al. 2002; Uchida et al. 2010; Mera et al. 2006), and increase PrRP expression following acute restraint stress (Mera et al. 2006). Stress exposure (water immersion/restrain) does not alter the extent of TH-PrRP co-localization (Maruyama et al. 2001). Interestingly, stress-induced Fos activation of PrRP neurons is attenuated in rats exposed to an overnight fast (Maniscalco et al. 2015; Maniscalco and Rinaman 2017), suggesting an interaction with metabolic signals. Notably, fasting-induced reduction in PVN Fos activation is also evident in GLP-1 neurons (Maniscalco and Rinaman 2013; Maniscalco et al. 2015). PrRP neurons are also sensitive to chronic stress, as repeated restraint stress increases NTS expression of PrRP mRNA (Toth et al. 2008). Central or intra-PVN injection of PrRP causes PVN Fos activation and ACTH and corticosterone release in a CRH-dependent manner (Mera et al. 2006; Seal et al. 2002), suggesting an excitatory role of PrRP on CRH release. Moreover, PrRP potentiates the action of a subthreshold dose of NE on ACTH release, amplifying the impact of catecholamines on CRH neurons (Uchida et al. 2010). Overall, it appears that PrRP works in conjunction with catecholaminergic neurons to modulate both acute and chronic HPA axis stress reactivity.

Restraint stress also increases Fos activation of NTS neurons containing nesfatin 1. These neurons are localized in the same region as GLP-1 cells and are Fos activated following acute restraint, suggesting that they are placed in proximity to known PVN projecting cell populations (Goebel et al. 2009; Goebel-Stengel et al. 2011). Nesfatin-1 increases calcium influx into CRH neurons in vitro, and elevates both ACTH and CORT when injected icv, suggesting that this peptide has the capacity to centrally activate the HPA axis (Yoshida et al. 2010).

There are numerous other neuropeptides localized to NTS neurons. Many of these are also expressed in extra-NTS stress-regulatory nuclei, and thus it is difficult to isolate specific effects of NTS neurons. An example is the stress-excitatory neuropeptide, NPY, which is present in TH-positive neurons in C2 (but not A2) neurons in NTS regions that likely project to the PVN (Everitt et al. 1984). However, NPY is also expressed in other PVN-projecting regions, such as the arcuate and dorsomedial hypothalamic nuclei (Chronwall et al. 1985). Thus, regulation of the HPA axis may be mediated by several sources, of which the NTS is one. The list of NTS peptides includes the opioid peptides dynorphin (Fallon and Leslie 1986), enkephalin (Khachaturian et al. 1983; Cunningham et al. 1991); and beta-endorphin (Palkovits et al. 1987); somatostatin (Priestley et al. 1991); and pituitary adenylate cyclase activating peptide (PACAP) (Hannibal 2002), among others. Most of these neuropeptides are capable of modulating HPA axis activity, but are made at multiple brain loci, leaving the connection with PVN activation tentative.

Regulation of the HPA Axis by the NTS: Glutamate

The PVN receives glutamatergic input from a number of different sources, including the NTS. There is evidence for a glutamatergic (vGluT2) projection from the NTS to the parvocellular PVN (Ziegler et al. 2012). As noted above, glutamate is co-localized with GLP-1 in PVN-projecting NTS neurons, indicating that the peptidergic projection may also signal via glutamate release (Zheng et al. 2015).

While the degree to which NTS glutamate modulates hypophysiotrophic PVN neurons is open to debate, the importance of glutamate in PVN drive is clear (Evanson and Herman 2015b). Direct infusion of glutamate in the PVN drives ACTH release and depletes median eminence CRH (Feldman and Weidenfeld 1997). The parvocellular PVN expresses NMDA, AMPA, and kainate ionotropic subunits (Herman et al. 2000), and physiological studies suggest that these receptors are functional (and excitatory). Indeed, local administration of a general ionotropic receptor antagonist (kynurenic acid) reduces HPA axis responses to acute restraint (Ziegler and Herman 2000).

The PVN also expresses group I and II metabotropic receptors. Our group has recently showed that local administration of an mGluR1 agonist inhibits HPA axis responses to restraint, whereas receptor blockade potentiates ACTH release (Evanson and Herman 2015a). We hypothesize that the inhibitory effect may be mediated by excitation of presynaptic GABA release.

Chronic stress causes marked changes in glutamate innervation of CRH neurons, suggesting a role in modulating HPA axis sensitivity. Notably, this effect is sex-specific: in males, vGluT2 innervation of PVN CRH cell somata and dendrites is markedly enhanced following two weeks of chronic variable stress (Carvalho-Netto et al. 2011). This regimen reliably produces sensitization of HPA axis responses to stress in males, suggesting a role for increased glutamateric drive. Conversely, vGluT2 innervation of the PVN is decreased, rather than increased in females (Carvalho-Netto et al. 2011). Since CRH co-localization studies have not been performed in females, the overall impact of this pronounced sex difference on hypophysiotrophic neurons is not immediately clear.

NTS Glucocorticoid Signaling and HPA Axis Regulation

Glucocorticoid signaling plays an important inhibitory ('feedback') role in limiting over-activation of the HPA axis and limit the potentially damaging effects of glucocorticoid overexposure. The NTS is rich in both the mineralocorticoid (MR) and glucocorticoid (GR) receptors, receptors that are bound by endogenous corticosteroids (Herman 1993). The GR binds stress levels of circulating glucocorticoids and is believed to be the primary receptor mediating glucocorticoid negative feedback regulation of ACTH release (De Kloet et al. 1998). Importantly, GR is richly expressed in catecholaminergic (Harfstrand et al. 1986) as well as non-catecholaminergic (e.g., GLP-1) neurons (Herman et al, unpublished observations) in the NTS, placing it in a position to modulate excitatory drive of the HPA axis by these cell populations. Consistent with this possibility, local implants of corticosterone inhibit HPA axis responses to stress. Conversely, implants of mifepristone, a

GR antagonist, has the opposite action, serving to enhance stress reactivity (Ghosal et al. 2014). Local implants of mifepristone also exacerbate HPA reactivity following exposure to either chronic variable stress or chronic restraint (Bechtold et al. 2009). Collectively, these data suggest that NTS GRs act as a site of HPA axis negative feedback, most likely by inhibiting NTS drive of neuroendocrine stress reactivity. Moreover, glucocorticoid signaling via the NTS may be an important control element limiting the impact of chronic stress on HPA axis drive.

Summary

The hindbrain NTS plays a prominent role in regulation of HPA axis responses to stressors, both in the acute and chronic domains (See Figure 1). Recent work has dispelled the notion that the effects of the NTS are primarily limited to catecholaminergic drive to systemic stressors. Work on non-catecholaminergic NTS systems reveals an important role for this region in drive of responses to psychogenic stimuli, as well as enhancement of HPA axis drive following chronic stressor exposure. The data suggest that there is a functional differentiation between neuronal subtypes in the NTS, likely associated with modality. Catecholaminergic neurons appear to drive HPA axis responses to homeostatic perturbations, perhaps associated with a role in reflex activation of the PVN. At least a proportion of the non-catecholaminergic neurons (e.g., GLP-1 cells) subserve PVN responses both homeostatic and psychogenic stimuli, suggesting connections to both reflex pathways and descending information, likely via descending limbic projections from regions such as the media prefrontal cortex and central amygdaloid nucleus (van der Kooy et al. 1984; Schwaber et al. 1980). Both NTS pathways use glutamate as a co-transmitter, and it is likely that both NE and GLP-1 are involved in modulating the magnitude of the excitatory response. Moreover, the role of NTS catecholamine neurons in chronic stress is more complex than previously believed, and may involve interactions with intermediary neuronal populations as well as serving to link autonomic drive with adrenal sensitivity.

In aggregate, the data point to an important role for this phylogenetically ancient nucleus in fine control of HPA axis responses to psychogenic stressors. Moving forward, it will be important to consider NTS driver systems when targeting intervention points for management of stress related disorders.

Acknowledgments

I am grateful for the inspiration provided by my friend and colleague Richard Kvetnansky, both for the studies contributing to this review and for my career in general. He was a major force in the stress field, and his leadership and support will be sorely missed. In addition, I would like to acknowledge all of the trainees and staff that have contributed to these studies, initiated at the University of Kentucky and continued on at the University of Cincinnati. Work on these studies was supported by MH049698 and MH069860.

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Figure 1.

Schematic of nucleus of the solitary tract (NTS) pathways regulating paraventricular nucleus (PVN) drive. Neurons in the PVN release CRH into blood vessels in the median eminence (ME), initiating the neuroendocrine cascade culminating in activation of HPA axis responses. Norepinephrine (NE) neurons in the NTS project to the PVN and act to excite HPA axis responses to systemic stressors. Under conditions of chronic stress, this projection appears to enhance stress reactivity by increasing adrenal sensitivity to ACTH, perhaps via effects on the sympathetic nervous system. A considerable population of NTS NE neurons co-express prolactin-releasing peptide, which may work in concert with NE to modulate PVN output. A separate population of NTS neurons express glucagon-like peptide 1 (GLP-1), along with glutamate (Glu). These neurons play a broader role in integration of acute HPA axis responses, serving to drive both psychogenic and system responses. In addition, these neurons appear to directly modulate sensitization of PVN responses to chronic stress.