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Neurochemistry of bulbospinal presympathetic neurons of the medulla oblongata

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Abstract

This review focuses on presympathetic neurons in the medulla oblongata including the adrenergic cells groups C1-C3 in the rostral ventrolateral medulla and the serotonergic, GABAergic and glycinergic neurons in the ventromedial medulla. The phenotypes of these neurons including colocalized neuropeptides (e.g. neuropeptide Y, enkephalin, thyrotropin-releasing hormone, substance P) as well as their relative anatomical location are considered in relation to predicting their function in control of sympathetic outflow, in particular the sympathetic outflows controlling blood pressure and thermoregulation. Several explanations are considered for how the neuroeffectors coexisting in these neurons might be functioning, although their exact purpose remains unknown. Although there is abundant data on potential neurotransmitters and neuropeptides contained in the presympathetic neurons, we are still unable to predict function and physiology based solely on the phenotype of these neurons.

Keywords

phenotype; neuropeptide; serotonin; catecholamine; thermoregulation; central cardiovascular control; sympathetic nervous system

1. Introduction

The neurons that innervate the spinal sympathetic preganglionic neurons (SPGNs) are termed presympathetic neurons. The SPGNs are the final common pathway for many reflexes important to homeostasis (e.g., maintaining blood pressure or body temperature at appropriate levels). Thus the presympathetic neurons are in the position to orchestrate these reflexes. Presympathetic neurons are located in the upper cervical spinal cord, medulla, pons and hypothalamus. This review will focus on the neurotransmitters and peptides associated with the presympathetic neurons located in the medulla oblongata and what the various phenotypes of these neurons might reveal about sympathetic control, with particular focus on cardiovascular control and thermoregulation.

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2. Bulbospinal Presympathetic Neurons

The sympathetic nervous system is responsible for “resting state” homeostatic functions as well as responding to stressful conditions such as exercise, disease or “fight or flight” situations. These functions are orchestrated through a complex network operating through SPGNs in the spinal cord that project to sympathetic ganglia. The neurons directly innervating the SPGNs are the presympathetic neurons. The presympathetic neurons are in the position to control a range of functions from blood pressure to bladder control. This review will focus on the presympathetic neurons in the medulla oblongata that control primarily cardiovascular and thermoregulatory functions.

Loewy and coworkers (1995a; 1997) found many groups of presympathetic neurons in the upper cervical spinal cord, medulla, pons and forebrain using the pseudorabies virus (PRV). The PRV is taken up by cells in a post-ganglionic target (e.g., adrenal gland or heart), replicates within the cell and then buds out and infects the next cell(s) in closest physical proximity, that is those that are synaptically connected (Card et al., 1990). This cycle of replication and infection continues through chains of synaptically connected neurons, thus revealing the sympathetic preganglionic neurons, sympathetic interneurons within the spinal cord (not normally referred to as “presympathetic” although they are directly antecedent to SPGNs) and the presympathetic neurons in the brainstem (as well as pons and forebrain). This method is useful in determining candidate presympathetic neurons and has identified the main groups of these presympathetic neurons in the medulla: the rostral ventrolateral medulla (RVLM) that contains the C1 cells, the rostral ventromedial medulla (RVMM) with the various raphe nuclei and the dorsal area around the medial longitudinal fasciculus/lateral tegmental field containing the C3 neurons. These groups of neurons will be discussed by primary phenotype and function.

3. RVLM Presympathetic Neurons

3.1. Catecholaminergic presympathetic RVLM neurons, the C1 cells

Fuxe and coworkers introduced the first technique that allowed identification of various monoaminergic neurons in brain and showed that some of these neurons had spinal projections (Carlsson et al., 1964; Dahlstrom and Fuxe, 1965). The C1 adrenergic neurons were first described by Hokfelt and coworkers (1974) who also suggested that C1 cells might be involved in vasomotor control (Bolme et al., 1974). The defining characteristic of these neurons is that they contain the catecholamine synthetic enzymes necessary for the production of epinephrine, i.e. tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT). The first demonstration that C1 neurons contain all the catecholamine synthetic enzymes was only relatively recently demonstrated by Phillips et al. (2001).

Reis and coworkers added substantially to the hypothesis that the C1 cells were presympathetic by identifying the spinally projecting PNMT-immunoreactive (ir) C1 neurons in the ventrolateral medulla and showing that PNMT terminals contact the SPGNs in rat (Tucker et al., 1987; Milner et al., 1988). Anderson et al. (1989) and Bernstein-Goral & Bohn (1989) replicated these findings of PNMT terminals in contact with SPGNs in the rat. Minson et al. (1990) added more evidence for C1 bulbospinal neurons and found that some of the PNMT innervation of the spinal cord comes from the more dorsal C2 and C3 adrenergic neurons (discussed in more detail below).

3.2. Other neuroactive substances present in C1 neurons

The RVLM C1 bulbospinal neurons contain several other neurochemicals including enkephalin (Stornetta et al., 2001), NPY (Blessing et al., 1987; Tseng et al., 1993; Stornetta et al., 1999), cocaine- and amphetamine-regulated transcript (CART) (Dun et al., 2002; Burman

et al., 2004), pre-pro-tachykinin (substance P) (Li et al., 2005) and calbindin (Goodchild et al., 2000). The colocalization of potential transmitter substances in the C1 cells has been previously reviewed by Pilowsky & Goodchild (2002). The most recently described potential transmitter found to be colocalized in the C1 neurons is the sympatho-excitatory neurochemical pituitary adenylate cyclase-activating polypeptide (PACAP) (Farnham et al., 2008).

To date, the phenotypic identification of cotransmitters in C1 neurons has yielded some limited predictive value on the projection pattern and conduction velocity of C1 cells with specific colocalized neuropeptides. One instance is that NPY tends to be in the C1 neurons projecting to the hypothalamus rather than to the spinal cord (only 10% of spinally projecting C1 cells contain NPY where 96% of C1 cells projecting to hypothalamus are NPY positive (Stornetta et al., 1999)). Although C1 neurons have a variety of conduction velocities, from a faster conduction suggestive of lightly myelinated axons to a slower conduction velocity associated with unmyelinated axons, there is some correlation of the colocalized peptide in C1 neurons with their conduction velocity. The C1-NPY neurons have a slow conduction velocity (Stornetta et al., 1999) while enkephalin containing C1 neurons tend to have a faster conduction velocity than non-enkephalinergic C1 cells (Stornetta et al., 2001).

We know something of the effects of some of these colocalized neuropeptides on SPGNs. For instance, using the technique of direct iontophoresis or pressure ejection onto identified neurons, substance P increased the firing rate of SPGNs (Gilbey et al., 1983; Backman and Henry, 1984; Dun and Mo, 1988; Backman et al., 1990). PACAP excites SPGNs (Lai et al., 1997) by directly potentiating NMDA-receptor-mediated responses (Wu and Dun, 1997). Although enkephalin itself has not been directly tested, another opiate, morphine, inhibits the activity of SPGNs (Guyenet and Stornetta, 1982) consistent with the generally inhibitory effect of enkephalin elsewhere in the CNS.

CART is excitatory to SPGNs (if one assumes that intrathecal application and measurement of blood pressure and heart rate are indicative of a direct effect on SPGNs), since CART applied intrathecally caused sympathoactivation at nanomolar concentrations, while at lower concentrations, CART dramatically increased the cardiovascular effects of intrathecal glutamate (Scruggs et al., 2005; Dun et al., 2007). The effects of intrathecal injections of NPY are variable depending on concentration (nanomolar concentrations produce pressor responses (Hassessian et al., 1990; Wager-Page et al., 1993) while sub-nanomolar concentrations produce depressor responses (Westfall et al., 1988; Chen and Westfall, 1993). The effect of NPY on SPGNs has not been directly tested. Elsewhere in the CNS, NPY has an inhibitory effect that is presynaptically mediated (Vezzani et al., 1999).

Although we know what the effects of the isolated substances on SPGNs might be, the combined actions of the colocalized neuropeptides in the C1 cells have not yet been fully determined. One possible explanation for the colocalized “inhibitory” peptides (e.g. enkephalin), is that when the system is undergoing maximal stimulation (given that peptides are released with higher stimulation frequencies (Lundberg and Hokfelt, 1983; Pernow et al., 1989; Iverfeldt et al., 1989; Drake et al., 1994; Vilim et al., 2000)), an inhibitory peptide could serve as an autoregulatory inhibitory feedback mechanism to bring the system back towards normal firing. Another hypothesis on the role of the colocalized neuropeptides put forward by Fuxe and coworkers (Zoli et al., 1998) is that the peptides are involved in “volume” transmission while the ionotropic transmitters are part of “wiring” transmission, i.e., normal synaptic transmission. This theory posits that because the locations of high affinity peptide receptors are often mismatched with the location of the peptide-containing terminals that the peptides would signal by diffusion from the terminals to the more distant receptor sites. Another variant on this theory was recently described by Leng and Ludwig (2008) where peptides have a low probability of release but can be released in large amounts under certain conditions and

thus “shout” rather than “whisper” like normal ionotropic transmission. Perhaps another explanation is that peptides could set a general membrane bias or tone where the synaptic transmission is accomplished by the ionotropic transmitter. However, these theories are still not tested in the C1 neuronal transmission to SPGNs and the exact role of the coexisting peptides in the C1 cells remains undecided at present.

3.3. Can the phenotype of particular combinations of colocalized substances in C1 neurons predict function?

So far, no phenotypic identification of the C1 cells including colocalized neuromodulators has yielded answers to other questions such as are particular subsets of C1 neurons specifically involved in the control of particular end organs. For example, which C1 cells are responsive to glucose levels (Ritter et al., 1998) and control sympathetic output to epinephrine-secreting adrenal chromaffin cells (Morrison and Cao, 2000) vs. which are the barosensitive C1 cells that regulate sympathetic outflow to blood vessels (Lipski et al., 1995; Schreihofer and Guyenet, 1997) and norepinephrine secreting adrenal chromaffin cells (Morrison and Cao, 2000)? The rostral/caudal location of a C1 neuron may be as good a predictor of its function as the specific colocalized peptide. A couple of examples: 1) Ritter (1998) found that the slightly more caudal C1 cells located closer to the A1 cells were likely to be glucosensitive, and 2) the more caudal C1 cells are likely to project to the hypothalamus (Verberne et al., 1999). McAllen has proposed the idea of the RVLM vasomotor area (termed the subretrofacial nucleus in the cat) being organized geographically with different subsets of vasomotor neurons driving, for example, skin vs. muscle sympathetic activity (McAllen, 1986; Dampney and McAllen, 1988; McAllen and Dampney, 1989; 1990; McAllen et al., 1995; 1997). These different geographic subsets of neurons have not been found in the rat, perhaps due to the relatively smaller area and denser packing of the neurons in the RVLM vasomotor area of the rat brain. One study (Polson et al., 1992) did attempt to phenotype the cells of the subretrofacial nucleus in the cat based on geographic region; however this study was anatomical and did not definitely characterize the physiological properties of any of the neurons.

3.4. Non-C1 RVLM presympathetic neurons

Although many of the bulbospinal neurons within the RVLM are C1 neurons, from 30-50% of the bulbospinal neurons in this area are non-C1 neurons (Ruggiero et al., 1994; Stornetta et al., 2002b). Guyenet and colleagues first brought attention to these non-C1 neurons in work on slices with bulbospinal cells labeled by retrograde tracers (Sun et al., 1988a; 1988b). The labeled cells were recorded in vitro and found to have intrinsic pacemaker properties that some believe to be part of the origin of sympathetic tone. None of these cells were immunoreactive for either PNMT or TH. These results may have been confounded by the recording technique where it is possible that the catecholamine enzymes were diluted by the contents of the intracellular recording pipette. However, the non-C1 presympathetic neurons were later confirmed by both intracellular and juxtacellular recording *in vivo* in adult animals (Lipski et al., 1995; Schreihofer and Guyenet, 1997), although they constitute a minority (~25-30%) of the presympathetic neurons in the RVLM. This relative percentage of C1 vs. non-C1 presympathetic neurons is in agreement with Ruggiero and coworkers who found that 72% of neurons in the RVLM retrogradely labeled from spinal cord injections were PNMT-ir (Ruggiero et al., 1994), (assuming that all RVLM spinally projecting neurons are presympathetic). The presympathetic C1 neurons generally have slower conduction velocities indicative of unmyelinated axons in agreement with the report from the Reis group (Milner et al., 1988) that most PNMT-ir axons in the IML region are unmyelinated. However, in another Reis collaboration, Morrison et al. (1988) reported some lightly myelinated C1 axons and found a range of conduction velocities for C1 neurons from slow to fast, corroborated by Schreihofer and Guyenet (1997). While the non-C1 presympathetic neurons generally have faster

conduction velocities (Schreihofer and Guyenet, 1997), whether or not a cell is catecholaminergic is not a prefect predictor of its conduction velocity.

3.5. Neuropeptides in non-C1 RVLM presympathetic neurons

Most of the peptides mentioned above that are present in C1 presympathetic neurons have also been found in non-C1 presympathetic neurons in the same general area of the RVLM. These include enkephalin (Stornetta et al., 2001), substance P (Li et al., 2005), NPY (Stornetta et al., 1990; Stornetta et al., 1999), CART (Burman et al., 2004) and PACAP (Farnham et al., 2008). While all these substances are found throughout the brain, substance P, NPY and PACAP in the medulla show a more restricted distribution, similar to that of the catecholamines, as far as the appearance in the area of the NTS, the lateral tegmental field and the RVLM. NPY also appears in the spinal trigeminal nucleus and substance P is also present in serotonergic neurons in the midline and RVMM. Some PACAP neurons are also found in the area of the midline raphe. Enkephalin has a much more widespread distribution throughout the medulla.

Burman et al. (2004) suggested that all presympathetic barosensitive neurons contain CART, whether or not they are immunoreactive (ir) for TH. This is based on c-Fos responses to nitroprusside injections (theoretically causing activation of presympathetic neurons by baroreceptor unloading but also potentially activating the neurons downstream of the barosensitive neurons) as well as direct recording and juxtacellular labeling of barosensitive neurons antidromically activated from spinal cord. While almost all c-Fos-ir neurons contained CART, CART was found in some neurons that did not express c-Fos so one must withhold some enthusiasm on the conclusion that CART expression marks a neuron as a barosensitive presympathetic neuron. It is possible that CART might mark presympathetic neurons with other sympathetic functions not related to barosensitive cardiovascular outflows. This has not yet been directly tested.

3.6. RVLM glutamatergic presympathetic neurons

Glutamate is most likely the agent of fast excitatory drive to sympathetic outflow controlling blood pressure. The origin of a glutamatergic presympathetic drive comes from the same area of the RVLM where the C1 cells are located (Dampney et al., 1982; Morrison et al, 1988; Reis et al, 1989; Morrison and Reis, 1991; Cechetto and Chen, 1992). Basil and Gordon (1993) noted that NMDA agonists were responsible for exciting SPGNs. Deuchars et al. (1995) demonstrated excitatory postsynaptic potentials (EPSPs) in SPGNs elicited by electrical stimulation of the RVLM and also showed evidence that this pathway was at least partly monosynaptic and glutamatergic, although the conclusions are limited by the fact that fibers of passage could also have been stimulated.

Morrison (2003) provided a brief review of this topic and noted that release of both glutamate agonists as well as electrical stimulation of the RVLM caused excitation of SPGNs and this excitation could be blocked with glutamate antagonists. Aicher et al (2000) also found NMDA receptors on neurons (likely SPGNs) postsynaptic to adrenergic terminals in the intermediolateral cell column (IML) in thoracic spinal cord. The finding that vesicular glutamate transporter type 2 (VGLUT2) was present in both C1 and non-C1 bulbospinal barosensitive neurons (Stornetta et al., 2002a; 2002b) suggested that the C1 neurons could be partly responsible for the glutamatergic presympathetic drive. Further support for this concept derives from the report of Nakamura et al. (2004b), who found VGLUT2 in asymmetric synapses onto choline acetyltransferase-ir neurons (SPGNs) in intermediolateral cell column of thoracic spinal cord. The VGLUT2 was present in DBH-ir (adrenergic or noradrenergic) terminals.

3.7. C1 and non-C1 neurons and cardiovascular control via catecholamines vs. glutamate

Whether or not the C1 cells participate in the control of blood pressure is a controversial topic which still has not been definitively proven. Lipski and coworkers (1995) found both adrenergic (TH-ir) and nonadrenergic bulbospinal barosensitive neurons. Guyenet and collaborators (1997) verified and extended this finding of both C1 and non-C1 presympathetic blood pressure sensitive neurons in the C1 area by recording barosensitive neurons antidromically activated from the spinal cord that were PNMT-ir. They also found non-PNMT-ir neurons that were presympathetic and barosensitive. Lesions of over 80% of the bulbospinal C1 cells result in only modest decreases in blood pressure (Madden et al., 1999; Madden and Sved, 2003), although some sympathetic reflexes are greatly attenuated (Schreihofer and Guyenet, 2000). Sved has argued that the C1 cells might not release epinephrine onto SPGNs (1989) (based on lack of HPLC detection of epinephrine in the spinal cord) and epinephrine as the “excitatory” transmitter of the presympathetic cells is controversial. Although catecholamines can produce an excitatory effect on SPGNs via alpha-1 receptors (Yoshimura et al., 1987a; 1987b; 1989; Inokuchi et al., 1992; Malhotra et al., 1993; Huangfu et al., 1994), catecholamines can also inhibit SPGNs via alpha-2 receptors (Franz et al., 1982; Guyenet and Stornetta, 1982; Inokuchi et al., 1992; Stornetta et al., 1995). Thus the catecholamines released by the C1 neurons, although not considered as “fast transmitters” and perhaps not responsible for the fast activation elicited by electrical stimulation from RVLM to SPGNs (Morrison & Reis, 1991), nonetheless affect the state of membrane polarization (the “gain”) and have a major influence on the degree of excitability of cardiovascular-related SPGNs and thus a major influence on blood pressure.

4. Dorsal Medullary Presympathetic Neurons: the C2 (?) and C3 cells

While much attention has been paid to the C1 adrenergic neurons of the RVLM as major regulators of sympathetic control of cardiovascular function, there are other catecholaminergic cell groups that project to the IML and may be presympathetic neurons. These presumed catecholaminergic presympathetic neurons include the C2 and C3 nuclei. Both C2 and C3 cell groups have been reported to project to the spinal cord (Sawchenko and Bohn, 1989; Minson et al., 1990; Farnham et al., 2008). However, whether the C2 neurons are indeed projecting to SPGNs is not certain. While Loewy and coworkers (Strack et al., 1989a; Loewy et al., 1994; Jansen et al., 1995b) report consistent findings on C3 neurons as presympathetic neuronal candidates, these same experiments show negligible C2 labeling from PRV injections into sympathetic targets. These findings call into question whether the C2 neurons are indeed presympathetic. However there is a report that C2 neurons could be presynaptic to parasympathetic pancreatic vagal motor neurons (Loewy et al., 1994). Certainly the C2 neurons could have an autonomic function due to their putative visceral inputs (Appleyard et al., 2007) (although these authors could not discriminate C2 from A2), inputs from the area postrema (Cunningham et al., 1994), activation by hemorrhage (Chan and Sawchenko, 1995; Buller et al., 1996; Dayas et al., 2001) as well as their projections to autonomic areas in the midbrain (Phillipson and Bohn, 1994) and hypothalamus (Cunningham et al., 1990). The C2 neurons could be in a position to coordinate sympathetic and parasympathetic systems. However, reports on the exact physiological role of the C2 neurons are still lacking. The C2 neurons are quite distinct from the C3 neurons. They differ in their morphology and in their projection patterns. Restricting reports to those that have confirmed co-localization of substances in spinally-projecting neurons, most of the dorsal medullary adrenergic spinally projecting neurons found to contain neuropeptides were the C3 neurons. NPY was found either rarely or not at all in the C2 neurons but abundantly in the C3 spinally projecting neurons (Jansen et al., 1995b; Stornetta et al., 1999). Enkephalin was absent in both C2 and C3 neurons (Stornetta et al., 2001). In agreement with the lack of enkephalin in C2 or C3 neurons, Loewy and coworkers found no (in C2) or extremely limited (in C3) immunoreactivity for the enkephalin-like peptide, MERGL, in neurons labeled with PRV trans-synaptically transported

from sympathetic targets (Strack et al., 1989b; Jansen et al., 1995b). Other than the lack of colocalized enkephalin, the C3 neurons seem very similar to the C1 neurons in terms of phenotype and projection characteristics and may be similar in terms of physiology. However, due to the sparsely scattered distribution of these neurons within the reticulum of the brainstem, experiments to determine their function as presympathetic neurons are difficult.

5. RVMM Presympathetic Neurons

5.1. Serotonergic presympathetic neurons

Carlsson et al. (1963) noted that levels of serotonin in rabbit spinal cord decreased dramatically in spinal segments caudal to a spinal transection, although this study could not identify the exact location of the descending source of serotonin. Bjorklund and Skagerberg (1979), using the fluorescent method for detection of indoleamines, determined that many of these spinally projecting serotonin neurons were located in the medulla oblongata by double labeling with a fluorescent retrograde marker. The existence of bulbospinal serotonergic neurons in the raphe pallidus (B1), parapyramidal area, obscurus (B2) and parts of magnus (B3) has been confirmed by many others (Bowker et al., 1981a; 1981b; Minson et al., 1984; Millhorn et al., 1987; 1989; Bowker and Abbott, 1990; Kwiat and Basbaum, 1990; Sasek et al., 1990; Jones et al., 1991; Johnson et al., 1993). However, the serotonin bulbospinal system is a good example of why the presence of a spinal projection does not necessarily identify a neuron as presympathetic. There is a plentiful serotonin innervation of the dorsal horn (Kwiat and Basbaum, 1992; Allen and Cechetto, 1994; Hökfelt et al., 2000; Geranton et al., 2008) involved in pain modulation (Mason, 2001). There is also a serotonergic innervation of motor neurons in the ventral horn (Zhan et al., 1989; Allen and Cechetto, 1994; Yates et al., 1999). Interestingly, the largest responses of serotonergic neurons in freely moving animals occur in relation to motor activity (Jacobs et al., 2002).

Bacon et al. (1990) used anterograde tracing and electron microscopy to show direct monosynaptic projections from RVMM to SPGNs. That some serotonin neurons in the RVMM (which includes the B1 and B3 serotonin groups) are indeed presympathetic was demonstrated by Loewy and coworkers with the PRV (Jansen et al., 1995b; Smith et al., 1998) and confirmed by others using similar methods (Stornetta et al., 2004; Nakamura et al., 2004a). Using the technique of anterograde tracing of serotonergic terminals on SPGNs from labeling in raphe also confirms the presence of serotonergic presympathetic neurons (Nakamura et al., 2004a). There is some evidence from double retrograde viral tracing studies that the same serotonin neurons that are presympathetic are also innervating motor neurons (Kerman et al., 2003; 2008) and may be involved in coordinating somatic and sympathetic efferents, for instance in the anticipation of exercise.

There is considerable evidence that at least some of the serotonergic presympathetic neurons have a role in thermoregulation (Morrison, 2004; Hodges et al., 2008) and some of these presympathetic thermoregulatory serotonin neurons also have the capacity for glutamate release (they contain VGLUT3 as discussed below) (Nakamura et al., 2004a). Much of this evidence derives from studies where PRV was injected in the major effectors of heat generation or loss in rats, i.e., brown adipose tissue or tail artery (Smith et al., 1998; Cano et al., 2003; Yoshida et al., 2003; Nakamura et al., 2004a). Serotonergic neurons in the RVMM were consistently labeled at short time courses suggesting that these neurons were synaptically connected to the SPGNs involved in regulating these temperature controlling outputs.

There are also suggestions that the serotonergic presympathetic neurons in the RVMM may have an excitatory effect on cardiovascular sympathetic outflow (Ma and Dun, 1986; Lewis et al., 1993; Pickering et al., 1994; Ramage, 2001). Serotonin iontophoresed onto SPGNs in thoracic cord of cats (Coote et al., 1981; McCall, 1983) excites these neurons. However,

serotonin seems to excite all SPGNs regardless of function (e.g., those innervating adrenal gland vs. those innervating other sympathetic ganglia (Backman et al., 1990)), so the serotonergic presympathetic neurons are not necessarily limited to those with vasomotor function. Serotonin could have a depolarizing effect on the post synaptic membrane via its action on $5HT_2$(2A) receptors (Ramage, 2001). Serotonin could also be a "neruomodulator" in changing the level of membrane polarization and thus setting the gain of the SPGN excitability much like the catecholamines (McCall, 1988).

Another explanation for sympathoactivation from stimulation in the raphe could be via glutamate. Madden and Morrison (2006; 2008) recently reported that spinal cord microinjections of serotonin can potentiate NMDA stimulated brown adipose tissue (BAT) sympathetic nerve activity. Whether glutamate could be coreleased from the serotonin terminals is controversial. The vesicular glutamate transporter, VGLUT3 is present both in bulbospinal serotonin neurons and in terminals making presumed excitatory contacts onto SPGNs (Nakamura et al., 2004a; Stornetta et al., 2005). However, VGLUT3 is also expressed by GABAergic neurons (Nakamura et al., 2004a; Stornetta et al., 2005) and, as noted above, serotonin is present in some GABA neurons. VGLUT3 is also found in bulbospinal neurons without serotonin or GABA (Nakamura et al., 2004a; Stornetta et al., 2005). Dissecting out the role of these different serotonergic neurons in sympathetic control is still a hot topic.

5.2. Presence of other neuroactive substances in RVMM serotonergic neurons

Bulbospinal serotonergic neurons and their terminals apposing SPGNs contain a variety of neuropeptides (for review see Hokfelt et al., 2000) including thyrotropin-releasing hormone (TRH) and substance P (Johansson et al., 1981; Helke et al., 1982; Appel et al., 1987; Nicholas et al., 1992; Johnson et al., 1993; Pilowsky et al., 1995; Hokfelt et al., 2000), somatostatin and enkephalin (Holets and Elde, 1983; Krukoff et al., 1985; Maxwell et al., 1996). Loewy has reported all of these neuropeptides in medullary presympathetic neurons using the PRV method (Jansen et al., 1995b). Both TRH and substance P increase the firing rate of SPGNs when applied by iontophoresis or direct application in slices (Gilbey et al., 1983; Backman and Henry, 1984; Dun and Mo, 1988) while somatostatin and enkephalin have inhibitory effects when tested on neurons in, for example, locus coeruleus (Inoue et al., 1988; Travagli et al., 1995) (although no one has specifically tested these peptides directly on SPGNs). Some of these cotransmitters may act synergistically with serotonin. Franck et al. (1989) reported that substance P could increase the evoked release of serotonin in the spinal cord. This would result in a feed forward effect; when conditions prevail for release of substance P (high firing as mentioned previously), even more serotonin would be released. The inhibitory neuropeptides could act as a brake on this system. Serotonin itself might change downstream excitability by acting on presynaptic 5HT1B "autoreceptors" to decrease release of serotonin (note that this 5HT receptor subtype has been found in spinal cord but not necessarily localized to SPGNs (Matsumoto et al., 1992)).

There is obviously much complexity inherent in the serotonin system for controlling sympathetic outflow, including whether serotonin is acting pre- or post-synaptically at SPGNs, which of the many serotonin receptors (Hannon and Hoyer, 2008) are activated and how the colocalized neuroeffectors might be interacting to synergize or negate the effects of serotonin. Research on serotonin and its cotransmitters at the level of the SPGNs is needed to determine these effects with greater precision.

5.3. RVMM inhibitory presympathetic neurons

Coote and Macleod (1974) had suggested that some bulbospinal neurons in the RVMM exerted an inhibitory influence on SPGNs. Electrical stimulation within the RVLM/RVMM produces inhibitory postsynaptic potentials (IPSPs) in SPGNs (Deuchars et al., 1997), although electrical

stimulation also excites fibers of passage as noted earlier, thus limiting the interpretation of the exact location of the IPSP-inducing neurons. Up to 50% of the contacts on SPGNs are inhibitory (Llewellyn-Smith et al., 1995; 1998), although some of these contacts come from spinal interneurons (Dun et al., 1992; Deuchars et al., 2005). Bulbospinal GABAergic neurons located in the RVLM/RVMM have been described by many different laboratories (Millhorn et al., 1987; Jones et al., 1991; Miura et al., 1994; Matsumoto et al., 1994). In his work with the PRV, Loewy described a large group of neurons in the RVMM that are presympathetic (Strack et al., 1989a). Some of these neurons are GABAergic and/or glycinergic. This was first illustrated by identifying GAD-67 mRNA in neurons retrogradely labeled from spinal cord fluorescent tracer injections (Stornetta and Guyenet, 1999). While a bulbospinal projection is necessary to identify a presympathetic neuron, it is not sufficient, and thus the subsequent use of PRV to identify presympathetic neurons was combined with GAD-67 and glycine transporter-2 (GLYT2) mRNAs in the virally infected neurons in the RVMM to identify these bulbospinal presumed inhibitory neurons as presympathetic (Stornetta et al., 2004). Some of the GABAergic bulbospinal neurons also could be glycinergic (i.e. express GLYT2: Stornetta et al., 2004), serotoninergic (i.e. express tryptophan hydroxylase-ir: Millhorn et al., 1987; Stornetta and Guyenet, 1999; Stornetta et al., 2004; 2005) as well as glutamatergic (i.e. express VGLUT3: Stornetta et al., 2005). It is possible that the sympathetic depressor area in the gigantocellular region of the RVLM described by Aicher (Aicher et al., 1994; 1995; 1997) might have its effects via these bulbospinal GABAergic/glycinergic neurons, although the proof for this is still lacking. There are a great number of presympathetic neurons in the RVMM whose function is still unknown and, according to the PRV results from Loewy and colleagues, this area remains one of the largest unexplored potential inputs to the SPGNs.

6. Summary

Many of the presympathetic neurons in the brainstem are monoaminergic, either adrenergic or serotonergic. Both of these neuronal groups have an extensive menu of colocalized peptides. This suggests a complex system for fine tuning sympathetic output based on which substances are released that could depend on the firing frequency or pattern of firing. There seems to be at least some specificity of anatomical location and phenotype for particular sympathetic outflows; midline serotonergic neurons are involved in thermoregulatory outflow to brown adipose tissue and tail artery where RVLM adrenergic cells are innervating SPGNs controlling cardiovascular sympathetic outflows, however, keep in mind that at least some serotonergic neurons also innervate SPGNs involved in cardiovascular function if the PRV data are considered. It is still unknown whether the same or different medullary neurons innervate these different pools of SPGNs. Figure 1 offers an overview of the data reviewed here.

One drawback to functional phenotyping based solely on a cell's content is that a cell's function will depend on projection patterns (both input and output), although some projection patterns might be predicted by combinations of colocalized neuropeptides (e.g., C1 neurons expressing NPY tend to project to the hypothalamus rather than the spinal cord). However we are still a long way from being able to predict exact function based on the phenotype of a neuron.

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Abbreviations

5HT, 5-hydoxytryptamine (serotonin)
Cal, Calbindin
CART, cocaine- and amphetamine-regulated transcript

DBH, dopamine-beta-hydroxylase
ENK, enkephalin
EPSP, excitatory postsynaptic potential
GAD, glutamic acid decarboxylase
GLY, Glycine
ir, immunoreactive
IML, intermediolateral cell column
IPSP, inhibitory postsynaptic potential
NPY, neuropeptide Y
PACAP, pituitary adenylate cyclase-activating polypeptide
PNMT, phenylethanolamine-N-methyltransferase
PRV, pseudorabies virus
RVLM, rostral ventrolateral medulla
RVMM, rostral ventromedial medulla
SPGNs, sympathetic preganglionic neurons
SubP, substance P
TH, tyrosine hydroxylase
TRH, thyrotropin releasing hormone
VGLUT, vesicular glutamate transporter

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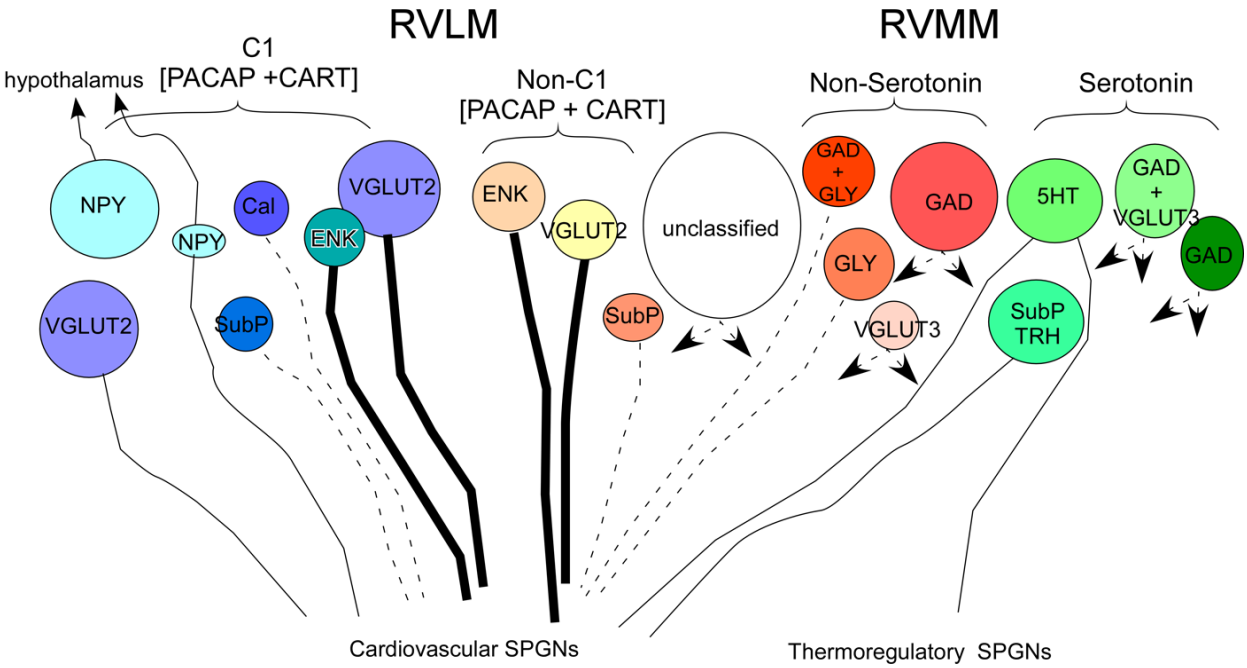

Figure 1.

Summary of data on neuronal phenotype, projections and axonal conduction velocity for presympathetic neurons. Thick lines represent fast conduction velocity (lightly myelinated axons), thin lines are slower conducting fibers (unmyelinated axons), dashed lines have unknown conduction velocity. Double pointing arrows represent neuronal populations with both cardiovascular and thermoregulatory SPGN targets. The larger circles represent the higher relative contribution to the spinal projection.

Abbreviations:

5HT, 5-hydoxytryptamine (serotonin); Cal, Calbindin; CART, cocaine- and amphetamine-regulated transcript; ENK, enkephalin; GAD, glutamic acid decarboxylase; GLY, Glycine; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; RVLM, rostral ventrolateral medulla; RVMM, rostral ventromedial medulla; SPGNs, sympathetic preganglionic neurons; SubP, substance P; TRH, thyrotropin releasing hormone; VGLUT, vesicular glutamate transporter.