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Article:

How sympathetic are your spinal cord circuits?

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New Findings

• What is the topic of this review?

This review focusses on the role of gap junctions and interneurones in sympathetic control at the spinal cord level

• What advances does it highlight?

The review considers the importance of these local spinal circuits in contributing to rhythmic autonomic activity and enabling appropriate responses to homeostatic perturbations.

Abstract

Sympathetic control of end organs relies on the activity of the sympathetic preganglionic neurones (SPNs) within the spinal cord. These SPNs exhibit heterogeneity with respect to function, neurochemistry, location, descending inputs and patterns of activity. Part of this heterogeneity is bestowed by the local spinal circuitry. Our understanding of the role of these local circuits including the significance of connections between the SPNs themselves, through specialised gap junctions, is patchy. This report focusses on interneurones and gap junctions within these circuits.

Gap junctions play a role in sympathetic control; they are located on SPNs in the intermediolateral cell column (IML). Mefloquine, a chemical that blocks these gap junctions reduces local rhythmic activity in the spinal cord slice and also disrupts autonomic control in the working heart brainstem preparation. The role that these gap junctions may play in health and disease in adult animals remains to be fully elucidated.

Presympathetic interneurones are located in laminae V, VII, X and the IML - those in lamina X are GABAergic and directly inhibit SPNs. GABAergic inputs onto SPNs exert their effects through activation of synaptic and extrasynaptic receptors which stabilise the membrane at negative potentials. GABAergic interneurones contribute to rhythmic patterns of activity that can be generated in the spinal cord since bicuculline reduces network oscillatory activity.

These studies indicate that local spinal cord circuitry is critical in enabling appropriate levels and patterning of activity in sympathetic outflow. We need to understand how these circuits may be harnessed in the situation of spinal cord injury.

Autonomic control of many end organs is achieved by the balance between parasympathetic and sympathetic outflows to each target organ. The output from the central nervous system in sympathetic control is through sympathetic preganglionic neurones that lie mainly in the lateral regions of the thoracolumbar cord. These neurones innervate either postganglionic sympathetic neurones or in some cases, such as the adrenal glands, directly innervate the end organ. Their level of activity is influenced by a number of descending, local and afferent inputs that together shape the final output from the spinal cord. We are interested in understanding the circuitry and cellular connections between neurones (via electrical synapses) that contribute to sympathetic outflow and in this report, will consider two aspects of these circuits; gap junctions and interneurones.

Gap junctions in sympathetic circuits

Gap junctions in the CNS play critical roles in coordinating activity in neurones. Gap junctions also exist between astrocytes to allow propagation of intercellular calcium waves between these cells, which leads to release of gliotransmitters that can ultimately activate postsynaptic neuronal receptors, in addition to neighbouring astrocytes (Orellana & Stehberg, 2014). Thus gap junctions may be pivotal in neuronal, astrocytic and glial-neuronal communication.

Gap junctions are formed from hemichannels in the 2 neighbouring cells; these hemichannels are themselves comprised of 6 connexin subunits (Figure 1A) and the expression patterns of these subunits convey the heterogeneity in their function. Gap junctions exist between SPNs; the major connexin subunit found is connexin 36, located along the dendrites and somata in the four main autonomic regions of the thoracic spinal cord (Marina et al., 2008). Functional evidence for these gap junctions comes from dual recordings between pairs of SPNs that exhibit truncated action potentials or "spikelets" in the recorded SPN that are due to the filtering of the fast components of the action potentials as they pass through the gap junctions of the electrically coupled SPNs (Nolan et al., 1999), figure 1B. Blockade of gap junctions reduces or even abolishes ongoing network oscillations in a spinal cord slice preparation, indicating that these electrical synapses may contribute to the overall co-ordinated output in the sympathetic nerves (Pierce et al., 2010, figure 2C. We have further shown that mefloquine, which can block connexin 36 containing gap junctions, reduces the sympathoexcitation due to chemoreceptor activation via supraspinal pathways in the working heart brainstem preparation (Lall et al., 2012, figure 2D). Mefloquine can have effects at other sites, including potassium and calcium channels (see (Pierce et al., 2010) for discussion) so a better approach is to use transgenic animals that better target these gap junctions. Indeed, our recent unpublished data using the connexin36 knockout mouse developed by Willecke and colleagues (Wellershaus et al., 2008) support the idea that these connexin 36 containing gap junctions may contribute significantly to the tight co-ordinated control of sympathetic activity. Although connexin 36 subunits are located at junctions between the majority of SPNs in the thoracic cord of adult rats (Marina et al., 2008), only a subset of around 25 % of SPNs actually demonstrate functionally active gap junctions in the form of spikelets (Logan et al., 1996) which may suggest some redundancy in the system. Those SPNs that exhibit spikelets show functional specificity since they likely innervate blood vessels in the skin (cutaneous vasoconstrictor SPNs) rather than muscle; gap junctions in these SPNs may contribute to the lower input resistances and long afterhyperpolarisations observed in these neurones (Stalbovskiy et al., 2014).

Gap junctions are becoming established as fundamental elements of neuronal and glial-neuronal communication but there has been controversy regarding whether they are an epiphenomenon of development in sympathetic control. There is little evidence of gap junctions in SPNs recordings in in vivo anaesthetised preparations where ongoing synaptic activity in the form of inhibitory and excitatory synaptic potentials is observed (Deuchars and Lall, 2015). However, connexin 36 immunoreactivity is abundant in adult rats, as evidenced by our own use of Cx36 reporter mice and immunohistochemical data (Marina et al., 2008) and spikelets have been reported in adult SPNs (see (Stalbovskiy et al., 2014) for discussion). The robust effects of the gap junction blocker mefloquine in six week old rats (Lall et al., 2012) suggest that they are functional at this age and are contributing to ongoing activity. Moreover, the incidence of spikelets did not change with age in the working heart brainstem preparation (Stalbovskiy et al., 2014). The contribution that gap junctions play in sympathetic control could be linked to pathology. After nerve injury in the adult rat, despite no alteration in the expression levels of connexin subunits, dye coupling (which is commonly used as an indicator of gap junctions) between somatic motoneurones is increased (Chang et al., 2000). Thus previously redundant gap junctions may now be activated to contribute to the neuronal plasticity associated with nerve injury or similar. Such a phenomenon could also apply in the sympathetic system. We therefore need to gain a better understanding of how the role of gap junctions may change according to the health or perhaps the age of an animal and whether manipulation of these gap junctions could be harnessed to reverse undesirable changes in the synchronicity of sympathetic outflow.

GABAergic interneurones and influences on sympathetic control

SPNs are not the sole neuronal subtype in the spinal cord contributing to sympathetic outflow, there is an abundance of interneurones that also contribute. Their presence and location was first elucidated by use of transneuronal tracers inoculated into specific end organs or sympathetic ganglia which led to infections of high numbers of non-SPNs, then classified as interneurones (Joshi et al., 1995;Cabot et al., 1994). These interneurones are located in laminae V, VII, X and within the intermediolateral cell region itself and some have activity that is either negatively or positively correlated to sympathetic activity, indicative of inhibitory and excitatory roles respectively (see (Deuchars, 2007;Deuchars, 2011)). Concerning excitatory interneurones, the peak in activity precedes the peak of renal nerve activity consistent with the neurones being antecedent to the SPNs (Schramm, 2006). One subgroup of interneurones that we have studied is located within lamina X (Figure 2A). These interneurones are GABAergic in nature and provide monosynaptic inhibitory inputs to SPNs (Deuchars et al., 2005). These interneurones may form part of the descending inhibitory control of SPNs that arises from the medial prefrontal cortex (Bacon & Smith, 1993). These or other spontaneously active GABAergic interneurones may also contribute to rhythmic network sympathetic activity; in the isolated spinal cord slice ongoing rhythmic sympathetic oscillations, produced by the local network of neurones and recorded from the intermediolateral cell column region, are attenuated by the $GABA_A$ antagonist bicuculline (Pierce et al 2010). In fact the GABAergic inputs onto SPNs, whether from local sources or from descending pathways, provide both tonic and phasic inhibition of sympathetic outflow. Ambient GABA in the spinal cord slice is continually activating $GABA_A$ receptors since bicuculline applications depolarise SPNs, while diazepam (which activates γ2 subunit-containing receptors that also contain either α1, α2, α3 or α5 subunits), but not zolpidem (a more selective benzodiazepine site modulator, which acts at α1, α2 and α3 subunit-containing receptors) enhances the inhibition (Wang et al., 2008), Figure 2B. This

type of tonic inhibition is normally due to activation of extrasynaptic receptors that are acutely sensitive to low GABA concentrations (Lee & Maguire, 2014) - in SPNs these receptors are partly comprised of α5 subunits, since it is this subunit that is present in PCR analysis of the intermediolateral cell column region and the pharmacology above fits with this profile. Tonic GABAergic inhibition is prominent in many different brain regions and is likely to contribute considerably to overall excitability, but this may be dependent on the membrane potential such that the contribution may be greatest at firing threshold potentials. This may be of functional relevance to SPNs, especially in situations where there is increased sympathetic activity that may be detrimental to health. In fact the idea that this tonic inhibition could contribute to overall blood pressure changes is supported by our understanding of the haemodynamic effects in humans of the two modulators used here. Diazepam, which enhances tonic inhibition, causes hypotension and reduces muscle sympathetic activity (Kitajima et al., 2004) while zolpidem, which was without effect on tonic inhibition, does not cause changes in blood pressure in humans (Cashman et al., 1987). Tonic inhibition may also play a role in limiting the frequency of oscillatory activity in other brain regions such as hippocampus (Mann & Mody, 2010), and a component of the effect of bicuculline of rhythmic network activity in the spinal cord slice may due to in part to blockade of the tonic current (Pierce et al., 2010). GABA$_B$ receptors also play a role in controlling SPN activity; these are present not only on the postsynaptic membrane but also on the presynaptic terminals arising from both descending and local GABAergic inputs to SPNs (Wang et al 2010).

Interneurones are likely to be key components of many of the descending pathways onto SPNs; stimulation of descending axons results in monosynaptic excitatory and inhibitory responses in interneurones (Brooke et al., 2004;Deuchars et al., 2001). Moreover, axons from the rostral ventrolateral medulla and corticospinal tract closely appose both SPNs and interneurones (Pan et al., 2005) and these may be influential in amplifying or modulating the response at a spinal level. However, it must be noted that the source of GABAergic influences may also be directly from supraspinal inputs, not just from the local interneurones, since there are GABAergic pathways originating in the RVLM and CVLM (Deuchars et al., 1997;Miura et al., 1994) These regions may lie just medial to the C1 and A1 groups of neurons since there was no overlap between GABAergic bulbospinal neurons and those that were TH-positive (Stornetta & Guyenet, 1999).

Interneuronal contributions to autonomic function after spinal cord injury

The contributions that interneurones make to overall sympathetic activity change considerably after injury to the spinal cord and these alterations may underlie some of the pathological autonomic symptoms observed after damage to the spinal cord. After spinal cord injury, changes in autonomic function occur that are distressing to the patient; paraplegic patients rate recovery from autonomic disturbances such as sexual, bladder and bowel function as a higher priority than recovery of other functions, such as the ability to walk (Anderson, 2004). Immediately following injury there is a decrease in sympathetic activity because of spinal shock (Karlsson, 2006). Associated with this is an exaggerated hypotensive response but with no reflex tachycardia, an indication of the loss of supraspinal pathways that would normally enable this reflex compensatory response. Two to four months post injury, autonomic dysreflexia occurs in patients where the injury is above the 6th thoracic level (Karlsson, 2006). If sympathetic activity is investigated more rigorously, it is clear that in some nerves, activity is maintained or even increased after acute spinal cord injury in the anaesthetised rat (Schramm, 2006), while in other nerves, the activity is decreased. Thus

interneurones must be providing drive to these sympathetic circuits in the absence of supraspinal pathways.

In sympathetic interneurones, ongoing activity is increased and a higher proportion of interneurones display activity correlated with renal sympathetic activity, likely due to loss of supraspinal inhibitory influences that normally dampen the levels of excitability (Schramm, 2006). There is evidence of local axonal sprouting neurones providing afferent inputs and local interneurones since there is an increase in the numbers of neurones immunoreactive for GAP43 (a marker for reactive sprouting) after spinal cord lesions (Weaver et al., 1997). Interneurones themselves have somewhat exaggerated responses to somatic stimulation (Krassioukov et al., 2002), which fits well with their elevated levels of excitability. Colorectal stimulation (simulating the situation that often causes autonomic dysreflexia) led to a significantly higher number of c-fos positive neurones in spinal cord injured rats compared to control, suggesting that the actual number of interneurones activated by these stimuli is increased (Hou et al., 2008). Whether these interneurones are excitatory or inhibitory was not determined but it is known that spinal transection results in a greater increase in numbers of inhibitory over excitatory inputs onto SPNs (Llewellyn-Smith & Weaver, 2001). Thus interneurones may play a prominent role in sympathetic control after spinal cord injury and may contribute to the exaggerated responses observed in autonomic dysreflexia.

It is therefore important to consider how one may restore normal sympathetic responses after spinal cord injury; many studies focus on the restoration of motor function but most patients desire to gain relief from autonomic dysfunction. In one recent study, brainstem derived neural stem cell grafts into the transection site of a complete transected spinal cord partially restored basal cardiovascular parameters and alleviated autonomic dysreflexia (Hou et al., 2013). This was partly due to these cells seemingly acting as functional relays to enable partial restoration of the supraspinal control of sympathetic circuits. Some of these new inputs may be onto interneurones as well as SPNs. Neural stem cells are also present in the spinal cord itself, since ependymal cells around the central canal can proliferate and become neurons, oligodendrocytes and astrocytes after injury (Barnabe-Heider et al., 2010) and it may be possible to harness the neurogenic potential of the spinal cord itself to restore function. Indeed we have recent unpublished evidence that we can manipulate the rate of proliferation of these stem cells by activation of specific receptors on the ependymal cells and will be exploring this further as a potential avenue for autonomic repair after spinal cord injury.

Conclusion

The control of sympathetic outflow from the spinal cord is a complex process involving contributions from descending, afferent and local inputs to produce the final output in the form of synchronised activity. In this report, I have focussed on just 2 aspects; gap junctions and interneurones but we need to fully understand the contributions of all these components in both health and disease to identify new avenues for manipulation to restore homeostasis.

Acknowledgements

Figure legends

Figure 1 - Gap junctions in SPNs
A. The schematic shows the components of the gap junctions formed between 2 cells.
B. Simultaneous recordings from two electrotonically coupled SPNs demonstrate conduction of membrane potential changes from cell 1 to cell 2. A series of rectangular-wave current steps (amplitude, -160, -80 and 40 pA; duration, 800 ms) injected into cell 1 elicited corresponding membrane potential responses in both neurones with action potentials in the directly recorded cell and spikelets in the coupled cell.
C. Network oscillatory activity recorded in the intermediolateral cell column region is reduced by the gap junction blocker mefloquine (given in the perfusate at a concentration of 1 µM), top trace shows the raw activity, middle trace is power and the bottom trace shows the autocorrelogram.
D. Sympathetic nerve discharge in response to chemoreceptor stimulation (arrows) is reduced in the presence of mefloquine. Recordings taken from the working heart brainstem preparation.
Taken with permission from (Nolan et al., 1999)B), (Pierce et al., 2010)C) and (Lall et al., 2012)D).

Figure 2 – Interneuronal influences on SPNs and the effects of stem cells grafts in spinal cord injury
A. Light micrographs of a spinal cord section showing the presence of glutamate decarboxylase (GAD-67) mRNA in a lamina X interneurone that was also transneuronally labelled with pseudorabies virus (PRV) after injection of the virus into the medulla of the adrenal gland. Using a setup as shown on the left, glutamate microinjected over the GABAergic interneurones elicited a hyperpolarising response in an SPN, which was antagonized by bicuculline.
B. Application of bicuculline depolarised this SPN, an effect which was enhanced by diazepam but not zolpidem. The inset shows the expression of α5 in the spinal cord and intermediolateral cell column (IML), suggesting that the response is mediated by extrasynaptic α5-containing GABA receptors on SPNs.
Reproduced with permission from (Deuchars et al., 2005)A), (Wang et al., 2008); B).

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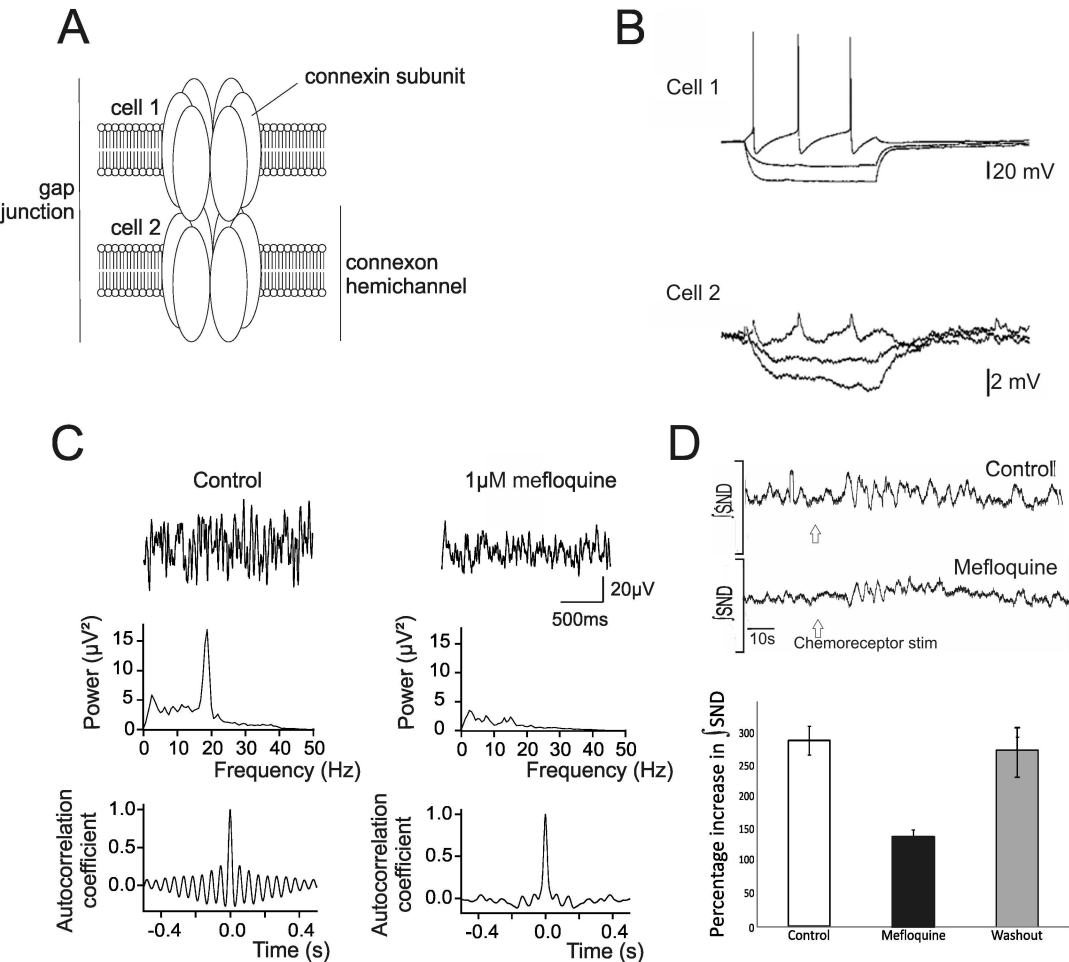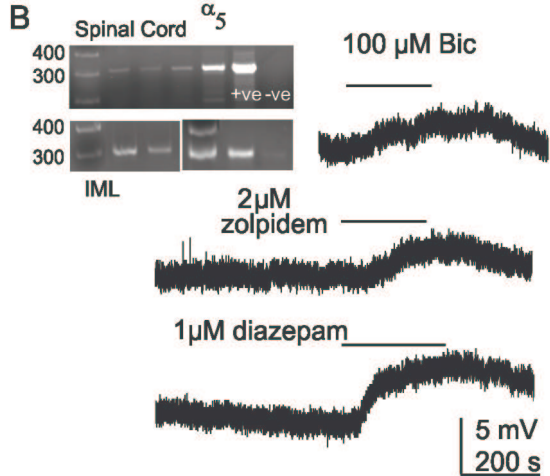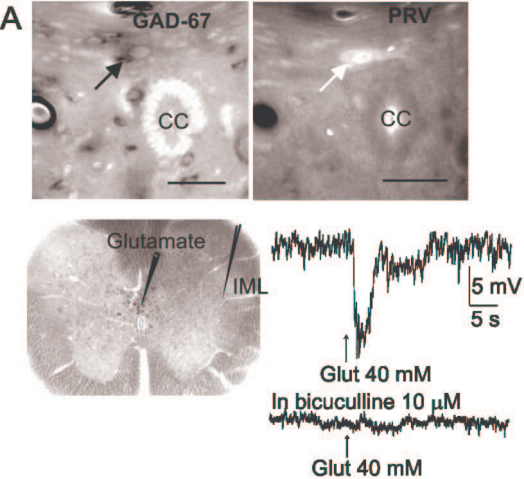
Figure 1

Figure 2