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Review Article

Synaptic Signaling in Sympathetic Vasoconstrictor Pathways and the Effects of Injury

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Abstract

Vasoconstrictor pathways through sympathetic ganglia appear to relay the pattern of signals originating in the central nervous system largely unmodified to the vasculature. Each preganglionic neuron distributes the centrally-derived signals to a number of postganglionic neurons (divergence) by way of single suprathreshold "strong" synapses. Strong synapses have a very high safety factor and differ from the multiple subthreshold or "weak" inputs in (a) releasing a much larger number of ACh quanta and (b) using characteristic Ca^{2+} channel subtypes to trigger their release. The most prominent subtype at strong synapses is resistant to all known Ca^{2+} channel antagonists. When postganglionic neurons are partially denervated, strong synapses are rapidly restored by sprouting of residual connections within the ganglia. This is the only function of weak synapses so far demonstrated. These singular properties emphasize the importance of strong synapses for guaranteeing synaptic transmission through sympathetic ganglia in vasoconstrictor pathways. [*Tzu Chi Med J* 2007;19(4):186–191]

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1. Introduction

Blood vessels throughout the body are surrounded by sympathetic noradrenergic nerve terminals whose activity leads to vascular constriction. This activity originates within the central nervous system (CNS) where action potentials discharge in preganglionic neurons after the integration of signals from many parts of the nervous system. These signals arise from segmental reflex inputs, intraspinal connections and descending supraspinal pathways. The discharge in most vasoconstrictor pathways characteristically exhibits cardiac rhythm as a result of the pulsatile baroreflex inhibitory input.

Preganglionic vasoconstrictor neurons lie along the thoracolumbar cord, mainly within the intermediolateral column and its lateral projection into the dorsolateral funiculus, and generally lateral to the preganglionic neurons involved with the control of visceral functions [1]. While the origin of excitatory drive to these neurons seems to be from a group of cells in the rostral ventrolateral medulla, the source

within the brainstem of the activity that drives these cells remains controversial [2,3], their final pattern of discharge is determined by the temporal and spatial summation of synaptic inputs impinging on the neuronal membrane [4,5]. These neurons, like most other central neurons, receive large numbers of excitatory (mainly glutamatergic) and inhibitory (GABAergic and glycinergic) inputs. The activity in each of these inputs effects only a very small change in membrane conductance. Threshold is reached when the membrane potential is depolarized by the amount of increased excitatory or decreased inhibitory input. Further, the frequency of action potential discharge is limited by a calcium-activated potassium conductance change that underlies an afterhyperpolarization lasting several hundred milliseconds. Thus, the average frequency of resting discharge of preganglionic axons in the anesthetized animal is 0.5–1 Hz [6]. Both ongoing and reflex discharge patterns of preganglionic axons are distinctive not only for anatomically distinct vascular beds, but also for the vessels in tissues with different functions [6,7].

Whereas the patterns of discharge of both pre- and postganglionic axons have been studied extensively, particularly by Wilfrid Jänig and his colleagues [6], and the responses of the peripheral vasculature to ongoing and reflex activity are the bread and butter of cardiovascular physiologists, the detailed cellular events during transmission of the signals along the peripheral pathways are only rarely considered. It has often been suggested that the pattern of activity is modulated at ganglionic synapses (e.g. [8]). Here, I will summarize what is known about transmission from pre- to postganglionic neurons in sympathetic paravertebral ganglia, which contain most vasoconstrictor pathways. In particular, I will address whether central vasoconstrictor commands are modified at ganglionic synapses and what happens at these synapses when some of their preganglionic inputs are lost by damage, e.g. to the spinal cord.

2. Transmission in sympathetic ganglia

As well as defining the thoracolumbar origin of sympathetic pathways, John Langley identified the synapses in peripheral ganglia on the way to the peripheral target organ [9]. He showed that postganglionic neurons were excited by nicotine painted on to the ganglion and reasoned that specific "receptors" on the neurons had been activated. Early studies of chemical transmission [10] subsequently identified acetylcholine (ACh) as the substance released from preganglionic nerve terminals. The postsynaptic receptors for ACh are now known to be nicotinic receptor-channels that, when activated, open to permit cations, including Ca^{2+}, to enter the postganglionic neuron. The properties of nicotinic receptors and their subunits in autonomic ganglia have been very extensively characterized [11,12]; the $\alpha3$, $\alpha4$, $\alpha5$ or $\alpha7$ subunits are most prevalent in sympathetic neurons, with the $\alpha7$ making the largest contribution to ACh-induced current. While application of exogenous ACh has been used to characterize these receptors, this does not reveal the mechanisms of synaptic transmission of nerve impulses. It is clear, however, despite evidence that purinergic synapses can form between two sympathetic postganglionic neurons *in vitro* [13], that ACh is the only functional neurotransmitter involved at synapses in intact sympathetic ganglia [14].

2.1. *In vitro studies*

The first electrophysiological studies of synaptic transmission between neurons were made in sympathetic ganglia [15]. Later, when intracellular microelectrodes were used [16], the responses evoked by preganglionic nerve stimulation consisted of several discrete excitatory postsynaptic potentials (EPSPs) that, when many were recruited, usually led to the discharge of a postganglionic action potential. Since then, although intracellular recordings have been made *in vivo* (see below), the details of synaptic microphysiology have largely been revealed in *in vitro* studies of isolated sympathetic ganglia. Ganglia can readily be dissected from an experimental animal with their connecting nerve trunks intact and then maintained *in vitro* in controlled ionic concentrations, oxygen tension and temperature that mimic the *in vivo* state. The membrane potential of the neuron soma can be recorded using a high resistance "sharp" microelectrode in the soma without significant disruption of the surrounding glia or synaptic connections. Passing small depolarizing and hyperpolarizing currents through the microelectrode reveals the cell's passive electrical properties. The cell input resistance of potentially vasoconstrictor neurons in adult rodent or guinea pig ganglia is ~150–200 MΩ and the input time constant is ~25 ms. Thus, specific membrane resistivity is ~25 $k\Omega \cdot cm^2$, much higher than that of most central neurons [17], and small brief conductance changes produce relatively large and prolonged passive voltage changes by opening of a few ACh receptor-channels.

Passing larger depolarizing currents through the microelectrode opens voltage-dependent channels in the soma membrane and triggers action potentials. In most neurons of the paravertebral chain, a depolarizing step initiates an early burst of action potentials and then silence (phasic discharge [18]). This is due to activation of M-type K^+ channels by depolarization and of Ca-dependent K^+ channels of both BK and SK types following Ca^{2+} influx during the action potential [18,19]. An afterhyperpolarization lasting

several hundred milliseconds ensues that can potentially delay the next action potential (and limit firing frequency) by preventing summation of small EPSPs from reaching threshold. However, in practice, summation is rarely observed (see below).

Preganglionic and postganglionic nerves to the preparation can be stimulated electrically to elicit EPSPs and antidromic action potentials, respectively. Graded stimulation of the incoming preganglionic axons reveals the number and size of individual synaptic inputs, at least for neurons that have only a few inputs or until they are obscured by the action potential. By hyperpolarizing the membrane to block action potential discharge, the amplitude of the EPSPs recruited at different stimulating voltages can be determined [20,21]. Using the single-electrode voltage clamp [22], a high resistance microelectrode can pass enough current to hold the somatic membrane voltage constant so that synaptic currents can be recorded and analyzed.

Studies of this kind (graded stimulation *in vitro*), made after reducing the number of preganglionic inputs by partial transection of incoming nerve bundles, or recording membrane activity *in vivo*, have revealed that each postganglionic neuron in adult ganglia (of mouse, rat, guinea pig, rabbit and cat [23–27]) receives one, occasionally two, rarely three, suprathreshold or "strong" preganglionic inputs and a number (2–15) of subthreshold or "weak" ones with EPSPs that range up to about 15 mV in amplitude. The total number of inputs is graded with animal size [28], suggesting that there may be >20 inputs per postganglionic neuron in humans. In addition, the number of inputs varies along the chain with fewer in more caudal ganglia [29]. Finally, while the total number of inputs directly parallels the number of primary dendrites [30], the synapses from each input are not restricted to a single dendrite. Most are formed on or close to the soma with a very low density of synapses distributed over the extent of the dendritic tree [31].

The synaptic currents underlying the EPSP evoked by each input show similar time constants of decay but a distinct cut-off in amplitude between weak and strong inputs, with strong inputs generating currents ≥1 (up to at least 10) nA and weak inputs <1 nA, the average being 0.3 nA. This implies that the number of quanta of ACh released at strong synapses is 3–30 times that at weak synapses. As the probability of release of ACh quanta is generally high (>0.5), even at weak synapses [32], it seems likely that the large quantal release from a strong input arises because it forms many more synaptic contacts on the postganglionic neuron than each weak input (Fig. 1). Because of the large number of released quanta, it can be very difficult to block the action potential using nicotinic antagonists at large strong synapses.

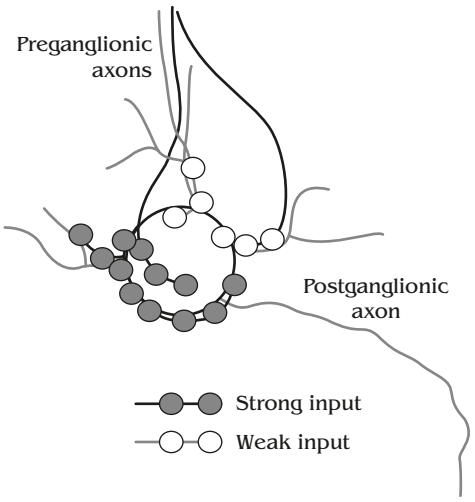

Fig. 1 — Diagram of likely configuration of the synaptic input to each paravertebral neuron, many of which are vasoconstrictor in function. Each neuron receives several preganglionic inputs and has a similar number of dendrites. Synaptic contacts are made on and close to the relatively large soma and at low density on the fine dendrites. One preganglionic axon makes many more synaptic contacts than the others. As each contact potentially releases one quantum of acetylcholine (ACh), the amount of ACh released by the strong input is large enough to guarantee the discharge of an action potential. Because of the low frequency of preganglionic discharge, this input is the only one that can generate action potentials to be conducted along the postganglionic axon to the peripheral vasculature.

The distinction between strong and weak inputs is emphasized further by the specific populations of Ca^{2+} channel subtypes on the preganglionic terminal membrane that are utilized for ACh release. Application of drugs and toxins selective for the various subtypes of Ca^{2+} channel (L, N, P, Q, R, T) revealed that weak inputs utilize N-type (40%), P-type (40%) and R-type (20%) channels, whereas strong inputs utilize only N-type (40%) and R-type (60%) channels [33]. The R-type channels appear to be insensitive to all Ca^{2+} channel antagonists so far identified. This means that currently known Ca^{2+} channel blockers are unlikely to affect transmission along most sympathetic nerve pathways.

From these studies, we can conclude that there are two qualitatively and quantitatively distinct types of ganglionic synapse in sympathetic vasoconstrictor pathways. One, sometimes two (rarely three) "strong" inputs always discharge action potentials with a large safety margin (like the neuromuscular junction); these synapses guarantee the onward relay of signals from their preganglionic origin in the spinal cord. Several "weak" small EPSPs arise from the activity of other preganglionic neurons but these seem very unlikely, individually, to trigger an action potential in

the postganglionic neuron. The weak inputs may simply be an ontogenetic "mistake", being left behind rather than withdrawn when functionally appropriate connections are established.

2.2. In vivo studies

Recordings have been made with microelectrodes in sympathetic ganglia in anesthetized animals, notably in Vladimir Skok's laboratory in Kiev [27,34] and later in Dale Purves' laboratory [26]. The advantage of these technically difficult experiments is that they demonstrate how the multiple preganglionic inputs identified in *in vitro* experiments interact during natural activity. In particular, the strong inputs are identifiable by their voltage configuration and their much greater amplitude than weak inputs during membrane hyperpolarization. This allows the behavior of these individual units to be followed during various interventions. Unfortunately, it is much harder to identify individual weak inputs because the time course of all subthreshold EPSPs is the same (being determined by the cell input time constant) and the amplitude of EPSPs arising from each input varies markedly with the spontaneous variations in quantal content.

Intracellular recordings from postganglionic neurons in the superior cervical ganglion (SCG) of anesthetized rats during reflex activation [21] have shown that the neurons discharge only when a strong input is activated. Even during high levels of ongoing and reflex activity, the probability of spatial summation of weak EPSPs appears extremely low, at least in the anesthetized animal. Thus, the postganglionic neurons fire at similar frequencies to the preganglionic ones (~1 Hz). When two strong inputs converge on the same postganglionic neuron, its firing frequency is increased and both signals are transmitted to the periphery. This occurs in ~25% of neurons in the adult rat SCG.

Because the number of postganglionic neurons is much higher than the number of preganglionic neurons (divergence) and each of the former receives a strong input, we generally assume that each preganglionic neuron makes strong inputs on several postganglionic neurons with the same function. The ganglia would then be a site of spatial amplification that distributes the central signal more widely. Alternatively, another less likely possibility is that a subclass of "strong" preganglionic neurons makes all the strong synapses, and the rest of the preganglionic neurons only weak ones; the latter would fail to send any signal to the periphery. It is tempting to suggest that such a set of weak connections functions only, for example, in times of emotional or physical stress, which we cannot investigate at the present time. It has been observed, however, that only strong inputs are discharged at high frequency during asphyxia [35].

3. Changes in connectivity in ganglia after damage to preganglionic neurons

When the thoracolumbar spinal cord is damaged, preganglionic neurons can be destroyed, leading to loss of some preganglionic inputs to neurons in associated ganglia. In my laboratory, David Ireland examined the consequences in L5 paravertebral ganglia of guinea pigs after transecting the paravertebral chain just proximal to the L4 white ramus, which is the most caudal preganglionic outflow in this species [36]. This retroperitoneal intervention was done to avoid damaging the spinal cord directly with subsequent distress for the animal. Neurons in L5 ganglia of guinea pigs on average receive one strong input and three weak ones, but only one in 10 of the strong inputs and fewer than one weak input originate in the L4 segment. Transection of the chain between L3 and L4 paravertebral ganglia leaves only this L4 contribution intact. After 3–5 weeks, however, nearly 60% of neurons received at least one strong input (a 7-fold increase and a significantly higher proportion than would have occurred by chance), whereas the mean number of weak inputs had only doubled. Thus, strong synapses were preferentially formed by sprouting of the original L4 population on to postganglionic neurons that lacked such inputs. Further, the newly formed strong synapses had normal characteristics as they operated only via N-type and R-type Ca^{2+} channels, like those in control ganglia.

Although it is not certain that all partially and completely denervated neurons would eventually have received a strong preganglionic input, it eventuated that many of the postganglionic neurons were very soon functionally reconnected. Probably the small population of remaining L4 preganglionic neurons would not have been able to supply enough sprouts to re-innervate all postganglionic neurons below the lesion. In similar experiments on the SCG, although T1 axons sprouted to innervate almost all neurons after removal of inputs from T3–7, the sprouting axons from C8–T2 supplied fewer contacts with each neuron (67%) than in controls [37], despite the relatively large number of sprouting axons.

Whether the new synapses between L4 preganglionic sprouts and postganglionic neurons were functionally matched is also unknown. Earlier studies of sprouting in the SCG after partial removal of preganglionic inputs from all segmental levels (C8–T7) demonstrated that selective connections with functionally appropriate outcomes were formed during sprouting from the residual inputs [38] and that new synaptic connections were stable although less extensive than the original innervation [39]. However, when the source of inputs was limited, as in Ireland's experiments, responses evoked from that source after

sprouting showed that control had spread to new functional groups (37). Thus, the formation of abnormal connections by preganglionic sprouts might help to explain the widespread activation of diverse functional sympathetic pathways when afferent inputs below a spinal cord injury trigger autonomic dysreflexia.

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