Neurotransmission Through Sympathetic Ganglia of Spontaneously Hypertensive Rats

Jeffery C. Magee and Geoffrey G. Schofield

Transmission of neuronal activity was assessed by recording preganglionic and postganglionic compound action potentials in superior cervical ganglia isolated from adult spontaneously hypertensive rats (SHR), Wistar-Kyoto (WKY) rats, and Wistar rats as well as young SHR and WKY rats to determine if previously observed alterations of membrane excitability, synaptic transmission, or both, have an effect on the transmission of preganglionic activity in SHR. Single stimuli induced more postganglionic neurons to fire over a wide range of preganglionic stimulation intensities in superior cervical ganglia from adult SHR as compared with those from adult normotensive controls. Short stimulation trains confirmed that SHR are able to maintain this greater number of active postganglionic neurons during low-frequency stimulation (1-20 Hz). However, by the end of a train of high-frequency stimulation (70-100 Hz) fewer neurons fired in ganglia from SHR compared with those from normotensive controls. These differences in transmission were not observed in the young rats. The results from the present study demonstrate that physiological frequencies of preganglionic activity are more effectively transmitted through sympathetic ganglia from adult SHR compared with those from normotensive controls, and this enhanced transmission through ganglia may contribute to the elevated sympathetic activity and the consequent hypertension seen in this model. (Hypertension 1992;20:367-373)

KEY WORDS • ganglia, sympathetic • hypertension, essential • sympathetic nervous system • rats, inbred SHR

An enhanced basal and environmentally evoked sympathetic nerve activity (SNA) has been implicated in the development and maintenance of the hypertension observed in experimental animal models such as the spontaneously hypertensive rat (SHR) as well as in essential hypertension itself. In support of this hypothesis, increased plasma catecholamine and dopamine-b-hydroxylase activity,1 enhanced SNA response to hypothalamic stimulation,2 enhanced cyclic nucleotide levels in sympathetic ganglia,3 sympathetic hyperreactivity to stressful stimuli,4 and direct recordings of exaggerated basal SNA have been reported in SHR.5,6

The neuronal alterations responsible for the enhanced SNA observed in SHR are still under study. The elevation of SNA could be the manifestation of an intrinsic membrane alteration that elevates the firing frequency of sympathetic neurons or of a modification in synaptic transmission that increases the probability of postsynaptic action potential generation, or both. For example, spike accommodation is compromised in adult and neonatal superior cervical ganglion (SCG) neurons from SHR.9,10 In addition, an increase in the stimulation-induced release of, and a hyperresponsiveness to, norepinephrine exist at the SHR sympathetic neuroeffector junction.11,12 Alterations in the concentration and turnover of catecholamines in many central cardiovascular areas and sympathetic ganglia have also been observed in SHR.4,13-15 These reports demonstrate that both membrane and synaptic alterations exist in the sympathetic nervous system of the SHR that could play a role in the elevation of SNA.

Ganglia represent a component of the sympathetic nervous system that have been studied extensively and provide an excellent model in which to study the transmission of nerve activity in SHR. Ganglia from SHR have been shown to possess a hyperexcitable membrane,9,10 decreased concentrations of the inhibitory transmitter dopamine, and an increased production of catecholamines in response to cholinergic stimulation.4,14,16 Thus, both membrane alterations and synaptic modifications have been observed in these ganglia; however, the effects of these alterations on transmission of neuronal activity through sympathetic ganglia have not been conclusively established. The present study was undertaken to determine if these alterations enhance the ability of preganglionic neurons to induce postganglionic neurons to fire action potentials and thereby contribute to the increase in SNA and consequent hypertension observed in SHR.

Methods

Preparation

Experiments were performed on 15 SHR, 15 Wistar-Kyoto (WKY) rats, and five Wistar rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) in strict accordance with...
institutional animal use guidelines. Age-matched adult (16–19 weeks) SHR, WKY, and Wistar rats as well as young (5–6 weeks) SHR and WKY rats were used in the present study. Wistar rats were used to control for the possibility that changes in ganglionic transmission observed between SHR and WKY rats could in fact result from a difference of WKY rats from the “normal rat population.” After conscious decapitation, the carotid bifurcations containing the SCG were removed and placed in iced Hank’s balanced salt solution. A ganglion was then removed from one of the carotid bifurcations, pinned to the floor of a recording chamber via the opened connective tissue sheath, and superfused with standard Locke’s solution at 1–2 ml/min.

Solution

The composition of the Locke solution (mM) was NaCl 136, KCl 5.6, NaH2PO4 1.2, NaHCO3 14.3, MgCl2 1.2, CaCl2 2.2, dextrose 11, and choline 0.03; the solution was equilibrated with a 95% O2–5% CO2 mixture and maintained at a constant temperature of 36±1°C throughout the experiment. Both left and right SCG were used.

Stimulation and Electrical Recording

Orthodromic stimuli were delivered through a suction electrode placed on the cut end of the cervical sympathetic trunk. Rectangular pulses (300 microseconds in duration) of varying magnitude and frequency were generated by an isolated electrical stimulator (S88, Grass Instrument Co., Quincy, Mass.). Extracellular recordings of preganglionic compound action potentials (CAPs) were obtained from suction electrodes placed en passant on a loop of the cervical sympathetic trunk, and postganglionic CAPs were recorded from suction electrodes placed on the cut end of the internal carotid nerve. AC recordings of CAPs were amplified by a custom-made differential amplifier (0.1 Hz–1.0 kHz) and displayed on a digital oscilloscope (2201, Tektronix, Beaverton, Ore.). Data were either stored on magnetic tape (3968A, Hewlett Packard, Palo Alto, Calif.) for later digitization and analysis or directly digitized using a 12-bit A/D converter (GW Instruments, Somerville, Mass.) and stored on the hard disk of a Macintosh II microcomputer (Apple Computer, Cupertino, Calif.) for later analysis. CAP magnitude was determined from the total area of the CAP calculated by the wave analysis program Igor, Wave Metrics, Lake Oswego, Ore. In the case of biphasic CAPs, the total area was calculated by summing the absolute value of the positive and the negative portions of the wave. The area of the CAP is considered to directly reflect the number of neurons contributing to the potential.17

The cervical sympathetic trunk probably contains some axons from postganglionic neurons in the inferior cervical ganglion. It is generally thought that these axons do not terminate in the SCG but course through it and travel with the other postganglionic axons to innervate their respective effectors.19 However, the contribution of these fibers to postganglionic CAPs has been shown to be minimal. Blockade of synaptic transmission by CdCl2 and the ganglionic blocker hexamethonium19 completely eliminates the postganglionic CAP. The effect of these fibers on the recorded preganglionic CAP, although difficult to ascertain, would also be expected to be minimal.

Single Stimulus Protocol

Input-output graphs were constructed from the preganglionic (input) and postganglionic (output) CAPs generated by single stimuli (0.25 Hz) the magnitude of which was increased from subthreshold to supramaximal (1–15 V). Both preganglionic and postganglionic responses were expressed as a percentage of maximal response. Figure 1A shows how both preganglionic and postganglionic CAPs increase in size as the voltage of stimulation is increased during stimulation at 0.25 Hz.

The largest CAP recorded from a ganglion was termed the maximal response and was considered to represent the size of the neuronal population that is available to fire action potentials in response to orthodromic electrical stimulation. Single stimuli at 0.25 Hz were sufficient to recruit all preganglionic axons to fire, thus producing the maximal input response. However, the maximal output response occurred during repetitive nerve stimulation at 40 Hz in all groups. All postganglionic CAPs were normalized to the maximum CAP assuming that the population of neurons that are activated during low frequencies remain active during 40 Hz stimulation.

Repetitive Stimulation Protocol

Repetitive stimuli were delivered as short trains of 10 supramaximal preganglionic stimuli ranging from 1 Hz to 100 Hz. Both preganglionic and postganglionic CAPs were recorded, and the areas of each CAP in the train were computed. Figure 1B and 1C show preganglionic and postganglionic CAP trains, elicited by the same 40 Hz stimulation. Supramaximal stimuli were used to ensure that the number of active preganglionic axons remained constant throughout the trains, and this was confirmed by monitoring the CAP magnitude. For quantification, the area of each postganglionic CAP was normalized to the maximal postganglionic CAP area for that ganglion.

Several precautions were used to minimize the impact of the relatively high frequency stimuli that were used: 1) The single stimuli used for generating input-output graphs were given at the beginning of each experiment before any repetitive stimuli were delivered. 2) Trains were kept relatively short (10 stimuli) and were separated by 1–1.5 minutes to reduce the effects of any muscarinic slow synaptic potentials.20 3) The relatively short nature of the trains precludes the induction of long-term or posttetanic potentiation,21–22 and the temporal separation also allowed ample time for any synaptic facilitation or depression to dissipate.24 4) The order in which different frequency trains were delivered was randomized. The area of the initial postganglionic CAP in all trains recorded from a particular ganglion did not change throughout the experiment, confirming that the preceding train had no lasting effect on synaptic transmission.

Statistics and Blood Pressure Measurement

Data are presented as mean±SEM. Statistical significance was tested using one-way analysis of variance combined with the Student-Newman-Keuls multiple range test. A value of p<0.05 was considered significant.
A nonlinear least-squares program based on the Marquardt-Levenberg algorithm was used to fit the input-output curves. The systolic blood pressure of every rat used in this study was recorded before experimentation with an indirect tail-cuff apparatus. Adult SHR with a systolic blood pressure less than 170 mm Hg and all other rats with pressures out of the normotensive range of 120-140 mm Hg were excluded from the study.25

Results

Transmission of Single Stimuli

Input-output curves were constructed to compare among the three strains of rats the efficacy with which SCG from the strains transmits neuronal activity. These curves describe a relation between presynaptic activity and intensity of the evoked postsynaptic discharge. The cervical sympathetic trunk was stimulated at 0.25 Hz, a frequency that produced no synaptic depression or facilitation. As the stimulation voltage was increased both the preganglionic and postganglionic CAPs increased in size (Figure 1A).

The curves obtained from adult ganglia (Figure 2A) indicated that as input increased output increased in an exponential fashion for both SHR and normotensive rats. The output of ganglia from adult SHR was larger for 25%, 50%, 75%, and 100% of maximum input when compared with the adult normotensive groups (Table 1). The input-output curve from a SCG from a young SHR was similar to that from a young WKY rat and did not show the exaggerated output that was found in the adult SHR (Figure 2B). Output from ganglia obtained from young SHR were not significantly different from the output recorded from ganglia removed from young WKY rats at 25%, 50%, 75%, or 100% input (data not shown).

Transmission of Repetitive Stimuli

The ability of SCG from the various strains to transmit repetitive activity was assessed using short trains of orthodromic stimuli. Trains of ten supramaximal stimuli were delivered to the cervical sympathetic trunk at varying frequencies (1–100 Hz), and the resulting postganglionic CAP trains (Figure 1C) were compared between groups. Preganglionic CAP magnitude remained within 5% of maximum throughout the train (Figure 1B) at all frequencies tested.

Low frequency orthodromic stimuli (1–10 Hz) evoked postganglionic CAPs from all groups that progressively increased to a plateau after three or four stimuli. At 1 Hz (Figure 3A) the CAP magnitudes from SHR ganglia were greater throughout the entire train when compared with the adult normotensive controls. This same pattern was maintained throughout a 5-Hz train (Figure 3B). However, the CAP magnitudes of normotensive
rats appeared to increase more at the beginning of the train than those from SHR that tended to reduce, but not remove the difference in CAP size between the SHR and normotensive groups by the end of the train. Thus, lower frequency trains revealed that ganglia from SHR had a larger CAP as a percent of maximum through the entire train.

High frequency trains (40–100 Hz) were given to determine if the differences observed in the CAP trains at lower frequencies could also be maintained at higher frequencies and to provide some insight into underlying mechanisms. Orthodromic stimulation of adult SHR ganglia at 40 Hz (Figure 4A) elicited postganglionic CAPs that again showed a larger initial magnitude than the adult normotensive strains. However, CAPs from the normotensive rats increased in magnitude during the train to such a degree that there was no difference in the CAP magnitude among the strains by the third stimulus. Figure 4B shows that at even higher rates of stimulation (80 Hz) the initial CAP magnitude of SHR ganglia was greater, but a dramatic decrease in CAP magnitude occurred over the duration of the train. On the other hand, CAPs in the normotensive groups initially increased to a peak after which CAP size decreased slightly. Thus, by the seventh stimulation the postganglionic CAPs from both adult normotensive groups were larger than those from the adult SHR ganglia.

At 40 and 80 Hz, no differences were observed between CAP magnitudes from young SHR and WKY ganglia. CAP magnitudes at 40 Hz began at 69.2±3.8% and 68.1±1.2%, increased to 89.4±1.4% and 90.6±1.9% by the fifth response, and finally reached 100.0±0.4% and 99.7±1.2% by the tenth response for SHR (n=8) and WKY (n=6) ganglia, respectively. At 80 Hz, CAP magnitudes began at 67.3±3.0% and 67.9±1.3%, increased to 85.0±5.4% and 76.8±3.2% by the fifth stimulation, and then dropped to 74.5±2.8% and 73.7±4.1% by the last for SHR (n=8) and WKY (n=6) ganglia, respectively. Thus, the normotensive young rats (SHR and WKY) had postganglionic CAP train profiles that appeared similar to the other normotensive controls (data not shown).

**Discussion**

The larger postganglionic CAP magnitude in adult SHR indicates that single stimuli activate a larger proportion of the postganglionic neuronal population that is recruitable by electrical preganglionic stimulation when compared with adult normotensive controls (WKY and Wistar rats). Moreover, the input–output graphs indicate that the efficacy of ganglionic transmission is enhanced over a wide range of preganglionic activation in adult SHR. In addition, SHR ganglia are able to maintain this larger proportion of active neurons throughout low frequency trains of preganglionic activity. Since a larger proportion of the postganglionic neuronal population in ganglia from SHR is activated by the initial stimulus in a train, there are fewer neurons available to be recruited during the remainder of the train. Thus, a smaller increase in CAP magnitude over the course of a train is observed in SCG from SHR when compared with WKY and Wistar rats. Stimulation at 40 Hz recruits all of the neurons available to fire action potentials so that during these trains ganglia from all groups have reached maximum output. During stim-

**Table 1. Postganglionic Output From Adult Rats (Percentage of Maximum)**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>25% of max</th>
<th>50% of max</th>
<th>75% of max</th>
<th>100% of max</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (n=5)</td>
<td>47.3±4.4*</td>
<td>70.2±3.1†</td>
<td>80.4±2.2†</td>
<td>83.4±3.3†</td>
</tr>
<tr>
<td>WKY (n=5)</td>
<td>28.8±2.3</td>
<td>44.2±2.1</td>
<td>57.0±1.2</td>
<td>66.1±3.2</td>
</tr>
<tr>
<td>Wis (n=5)</td>
<td>36.1±3.7</td>
<td>50.8±2.2</td>
<td>59.0±1.2</td>
<td>64.3±3.5</td>
</tr>
</tbody>
</table>

Individual output values for each ganglion were interpolated from best fits of the input–output data to an exponential function. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Wis, Wistar rats. Data are presented as mean±SEM.

*p<0.01; †p<0.001.
ulation at 80 Hz, the decrease in CAP magnitude seen in SHR ganglia indicates that the proportion of this population that is able to fire actually decreases, such that by the end of the train normotensive rats have significantly more neurons firing than SHR. Thus, in ganglia from SHR, greater numbers of postganglionic neurons are induced to fire as the frequency of stimulation is increased (1–40 Hz), but as stimulation frequency is increased still further to 80 Hz, the numbers of neurons firing during stimulation actually decreases. This decrease in the number of neurons firing at the highest frequencies was not observed in SCG from the normotensive rats.

This reversal from increased to decreased efficacy suggests a possible mechanism underlying the modified transmission of neuronal activity through SCG from SHR. The regulation of synaptic efficacy and neuronal input–output relations is complicated and encompasses properties of both the presynaptic and postsynaptic neurons. The active and passive membrane properties that control action potential generation play a role in the regulation of the transmission of neuronal activity.24 An alteration of spike accommodation has been shown in SCG neurons of SHR.25-26 A larger percentage of these neurons fire multiple action potentials rather than firing only one or two, as would be expected for sympathetic neurons, during a 400 millisecond current injection. Intracellular recordings from these SHR neurons have shown high frequencies of firing during current injection, approaching 30 Hz at room temperature27 and 80 Hz at 35°-36°C (unpublished observations from our laboratory). If the enhanced ability of SHR neurons to fire repetitive action potentials played a significant role in the transformation of preganglionic activity into postganglionic activity, one would expect an increase in transmission over all frequencies tested compared with normotensive strains. However, the number of postganglionic neurons that continue to fire action potentials throughout high frequency preganglionic activation is significantly reduced in ganglia from SHR. This surprising observation would seem to indicate that the altered ability of SHR ganglia to transmit neuronal activity does not result from the enhanced postganglionic membrane excitability.

Both presynaptic and postsynaptic factors involved in synaptic transmission also play a major role in the transmission of neuronal activity.27 Alterations in synaptic transmission have been reported for various synapses in the SHR. For example, increased release of norepinephrine from sympathetic terminals27 and decreased concentrations of the inhibitory transmitter dopamine in the SCG4 have been reported. Both
these observations point to synaptic alterations in the SHR that would lead to an enhanced efficacy of transmission at that particular synapse. Following this line of evidence, an increase in transmitter release from preganglionic neurons could explain the results presented here. Specifically, an increase in quantal content has been shown to produce larger excitatory postsynaptic potentials, which due to transmitter depletion displays decreased facilitation and greater depression during trains of activity.36-39 Also, the previously reported decreased dopamine concentrations could play a role in the enhanced transmission observed at low frequencies by decreasing the inhibitory influence of dopamine on the postganglionic neurons. However, one would also suspect that this decreased inhibition would also be present at the higher frequencies. Alternatively, a decrease in transmitter breakdown after anticholinesterase treatment has been shown to produce excitatory postsynaptic potential modifications that result in CAP trains that appear very similar to those recorded from SHR ganglia.30 Thus the altered ability of SHR ganglia to transmit neuronal activity is more easily explained on the basis of a modification (or modifications) in synaptic transmission rather than a hyperexcitable postganglionic membrane. However, further intracellular studies of various synaptic parameters are needed to confirm this hypothesis.

Normal physiological rates of preganglionic activity are typically within the 1–6 Hz range,21-33 although firing rates up to 15–40 Hz have been observed during extreme conditions such as hypoxia and hypercapnia.32 The input–output graphs demonstrate that single stimuli activate a greater proportion of the available postganglionic neuronal population in ganglia from adult SHR than in those from WKY and Wistar rats over a wide range of preganglionic axon activation. CAP trains generated by low frequency stimuli indicate this larger proportion of active neurons maintained during normal physiological levels of activity. Thus, similar levels of preganglionic activity in vivo would lead to the activation of more postganglionic neurons in the sympathetic ganglia of adult SHR when compared to adult normotensive controls. This increased amplification in the number of neurons involved in sympathetic outflow could lead to the cardiovascular and renal alterations which may underlie the hypertension seen in this model.34-39

An enhanced efficacy of impulse transmission was found in the adult SHR but not in young rats. Since this alteration was not observed in SHR at an age where the elevation in blood pressure is not completely established (5–6 weeks), it is possible that this modification does not participate in the initiation of the hypertension. Having said this, it is still conceivable that the alteration in transmission could develop later than 5–6 weeks of age and still precede the establishment of hypertension. However, a conservative interpretation of the data is that the alteration of the transmission of neuronal activity observed in SHR ganglia would contribute to the maintenance of hypertension through the potentiation of SNA rather than to its initiation.

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References

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