Loss of Accommodation in Sympathetic Neurons from Spontaneously Hypertensive Rats

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SUMMARY  Synaptic transmission and membrane properties of sympathetic neurons in superior cervical ganglia of spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto rats (WKY), and Sprague-Dawley rats (SD) were investigated in vitro by extracellular and intracellular recording. The sympathetic neurons of SHR showed an atypical loss of spike accommodation. The spike discharge was insensitive to the sodium channel blocker tetrodotoxin, but it was reversibly blocked by a variety of calcium antagonists. The loss of accommodation in the neurons of SHR was not due to a loss of M-current, a potassium current involved in controlling spike frequency adaptation in sympathetic neurons. Superfusion of ganglia of SHR with muscarine (10 μM), which suppresses M-current and leads to a loss of accommodation, potentiated the repetitive discharge. In the presence of muscarine the current-voltage curves in neurons of SHR and SD were shifted to similar extents. Resting membrane potentials of neurons of SHR and WKY were consistently depolarized as compared with neurons of SD. Synaptic efficacy through the ganglia of SHR, assessed by extracellular recordings of presynaptic and postsynaptic compound action potentials at 0.25 Hz stimulation, was elevated when compared with the ganglia of WKY, but was similar to that of the ganglia of SD. These results indicate that strain differences should be considered when attempting to attribute changes in sympathetic neuron membrane properties to hypertension. The sympathetic neurons of SHR appear to have lost their accommodative properties and might possess an exaggerated calcium conductance. This calcium conductance may explain the augmented calcium-dependent release of norepinephrine during sympathetic nerve stimulation in the SHR. (Hypertension 7: 268–276, 1985)

KEY WORDS  • membrane properties • repetitive firing • calcium conductance • normotensive strains

EXAGGERATED activity in the sympathetic nervous system in the Okamoto-Aoki strain of spontaneously hypertensive rats (SHR) has been implicated in both the development and the maintenance of hypertension in this model.1,2 Numerous studies with the SHR have shown that both central and peripheral neurogenic mechanisms are contributory factors, although they may not account for all the elevation in peripheral vascular resistance.2 There is an increased basal sympathetic tone in visceral and nonvisceral sympathetic nerves, and an elevated sympathetic discharge and afterdischarge during and following stimulation of the posterior hypothalamus.3,4 In addition, there is a vasoconstrictor hyperresponsiveness in several organs following sympathetic nerve stimulation,5 an increased release of norepinephrine,6 and an exaggerated sensitivity to norepinephrine in arterial muscle.7 Thus it would appear that both presynaptic and postsynaptic sympathetic functions are altered in this model of hypertension.

The basis for the altered sympathetic nerve activity observed in human hypertension or in the SHR remains obscure. Enhanced sympathetic nerve activity could reflect larger amounts of transmitter released from preganglionic neurons, which would result in the recruitment of larger numbers of autonomic neurons. Additionally, potentiated transmitter release would activate more muscarinic and noncholinergic receptors on ganglion cells, which would produce enlarged slow synaptic potentials and afterdischarges.8,9,10 Changes in sympathetic activity could also arise from an alteration in a variety of membrane properties that control firing fre-
frequency of the postganglionic neurons. The present study was undertaken to gain insight into the cellular nature of the hyperactivity occurring in sympathetic postganglionic neurons in hypertension. We compared both active and passive membrane properties from postganglionic neurons of the in vitro superior cervical ganglion of SHR with those from the genetically related normotensive Wistar-Kyoto rats (WKY) and the normotensive Sprague-Dawley rats (SD). Two different normotensive strains were used because of the recurring question of the appropriateness of WKY as a control (see refs. 2 and 15 for a discussion of this issue). Our results show that the postganglionic neurons of SHR have a lower resting membrane potential and possess a more prevalent regenerative calcium conductance than do autonomic neurons of SD and that synaptic transmission through the ganglion of SHR is heightened as compared with that through the ganglion of WKY, but is similar to the transmission through the ganglion of SD.

Materials and Methods

The isolated superior cervical ganglion from 7- to 13-week-old SHR, WKY, and SD (Taconic Farms, Germantown, NY) were used in these studies; younger and older animals were examined occasionally. Intracellular recordings were obtained from single superficial neurons from the superior cervical ganglion bathed in flowing Locke solution at room temperature (22–25°C). The neurons were impaled with a single microelectrode (DC resistances, 40–80 MΩ) filled with 4 M potassium acetate, neutralized to pH 7 with glacial acetic acid. Electrodes were capable of passing up to 1 nA of current (tested before and after impalement) without appreciable rectification; at higher injecting currents, corrections for rectification were occasionally necessary. Membrane potential was recorded with respect to ground with a high input-impedance electrometer (WPI, New Haven, CT). Bipolar signals from pulse generators were mixed through the active bridge circuit of the electrometer to provide known amounts of current through the microelectrode while recording neuronal potentials. Voltage recordings were displayed and photographed on a storage oscilloscope (DC 10 kHz) and on a chart recorder (Gould, Inc., Cleveland, OH). Data were also stored for analysis on magnetic tape (Hewlett-Packard Instrumentation FM tape recorder, type M3960; Palo Alto, CA).

Measurement of the resting membrane potential, spike amplitude, spike duration, spike threshold, and the amplitude and duration of the hyperpolarizing afterpotential was made as follows: resting membrane potential, the difference between electrode potential in the bath and in the neuron 5 minutes after impalement; steady state input resistance, the voltage change, after 300 msec, in response to a hyperpolarizing constant current pulse (100 pA, 350 msec); spike amplitude, the difference between resting and peak membrane potential; spike threshold potential, an estimate of the membrane potential of the onset of the upstroke of the action potential; cell time constant (at resting potential), the time for the membrane potential to change 63% toward the peak potential during a hyperpolarizing constant current pulse; measurements of the amplitude and duration of the hyperpolarizing afterpotential were made as previously described by McAfee and Yarowsky. Specifically, the amplitude of the afterpotential was measured as the difference between resting potential and the membrane potential at the peak of the afterpotential. Its duration was determined by measuring the time from the onset of the afterpotential until its return to resting potential. More than 100 postganglionic neurons were studied. These cells had a minimum spike height of 70 mV, a minimum input resistance of 40 MΩ, and a minimum resting potential of −40 mV. Mean values (±sem) are presented in the tables. In all cases the 0.05 confidence level was accepted as significant (analysis of variance). The steady state input conductance (Gₛ) was calculated as the reciprocal of the steady state input resistance. Current-voltage curves were constructed from the steady state values of the electronic voltage transients obtained by passing depolarizing and hyperpolarizing current pulses (100–800 pA in intensity). The steady state slope conductance (Gₛ) versus membrane potential was calculated from tangents drawn to the current-voltage curves (see ref. 23). The frequency of repetitive spiking during the intracellular depolarizing current pulse was determined by taking the number of action potentials during the first 100 msec and the number of action potentials during the last 300 msec of a 400-msec pulse.

Extracellular recordings of the preganglionic and postganglionic compound action potentials were obtained by placing suction electrodes on the internal carotid nerve (preganglionic) and en passant on a loop of the cervical sympathetic trunk (preganglionic). The cut end of the preganglionic nerve was placed within a third suction electrode for preganglionic stimulation. The preganglionic nerve was stimulated with rectangular pulses (300 μsec duration) at a rate of 0.25 Hz. Recordings of preganglionic and postganglionic action potentials were amplified by a differential AC preamplifier (0.1 Hz–1 kHz). Input-output curves were constructed by measuring the areas above baseline of preganglionic and postganglionic responses while the stimulus voltage was varied from threshold to maximal. Both preganglionic and postganglionic responses were expressed as a percentage of maximal response. The input-output curves from a given strain were normalized in the following manner: (1) each input value was placed into one of ten equal-sized groups according to its percentage of maximum response and (2) the average output was determined for each percentage input group and plotted as a mean ± SEM.

The composition of the Locke solution was: 136 mM NaCl, 5.6 mM KCl, 1.2 mM NaH₂PO₄, 14.3 mM NaHCO₃, 1.2 mM MgCl₂, 2.2 mM CaCl₂, 11 mM dextrose, and 0.03 mM choline; equilibrated with 95% O₂, 5% CO₂ throughout the experiment. Drugs were added by switching the superfusion to a drug-containing Locke solution. Measurements of various mem-
brane properties usually commenced 3 minutes after switching to a new Locke solution. Because of the relatively high flow rate (1.0–2.0 ml/min) and small bath volume (approximately 100 μl), this time period was deemed satisfactory to reach a new steady state in the recording chamber.

Reagent grade chemicals were used throughout. Tetrodotoxin (TTX) and tetraethylammonium bromide (TEA) were obtained from Sigma Chemical Company (St. Louis, MO). (+)Muscarine chloride was a gift from Dr. Dermont B. Taylor.

Arterial blood pressure in animals anesthetized with chloral hydrate (400 mg/kg), given intraperitoneally, was measured from the right femoral artery by means of a pressure transducer (Statham, Gould, Inc., Cleveland, OH) and displayed on a polygraph (Grass Inst., Quincy, MA).

Results

Membrane Properties of Postganglionic Neurons

Input resistance and spike parameters (amplitude, threshold, and hyperpolarizing afterpotential amplitude and duration) in neurons of SHR showed no significant differences when compared with similar measurements from neurons of normotensive SD or WKY (Table 1). By contrast, intracellular recordings from the postganglionic neurons of the SHR revealed an atypical phenomenon; namely, a repetitive action potential discharge during intracellular depolarization with current pulses ranging in intensity from 100 to 500 pA. Anodal-break spikes also were elicited following intracellular hyperpolarization over the same current range (Figure 1). Based on our rigid criteria for acceptable cell penetrations, the occurrence of multiple spikes and anodal-break spikes in the postganglionic neurons of the ganglion of SHR was observed in more than 85% of the postganglionic neurons examined (72 of 83 cells in 22 ganglia, 22 animals).

Multiple spiking was seldom observed in normotensive strains of rats (SD or Wistar), either in our studies or in those of Kiraly and Dolivo. Multiple spiking was measurable in the genetically related normotensive WKY, but it occurred in only about half the neurons studied, and its intensity, pattern, and duration were very different (Table 2). For instance, neurons of WKY rarely could maintain repetitive discharge beyond the first 100 msec of a depolarizing current pulse (Figure 2). When repetitive activity was examined quantitatively in the postganglionic neurons of SHR, two distinct rates of spiking were observed: a high-frequency discharge (approximately 29 Hz) occurring at the onset of the depolarizing current pulse and a slower maintained discharge (approximately 11 Hz). By contrast, only half of the normotensive ganglia of WKY revealed multiple spiking. When the multiple spiking was present, its initial and maintained rates were 22 and 2 Hz respectively (32 of 63 cells in 22 ganglia, 22 animals). When neurons from ganglia of SD yielded a second spike, they invariably did so during the first 100 msec of a 400-msec depolarizing pulse. Thus a major distinguishing feature between autonomic neurons of SHR and those from normotensive WKY or SD appears to be their loss of accommodation (Figure 3).

![Figure 1. Intracellular records from autonomic neurons of the superior cervical ganglion of SHR reveal a normal amplitude and duration of the hyperpolarizing afterpotential (A), multiple spikes during a depolarizing current pulse (C), and anodal-break spikes (B). The resting membrane potential was -52 mV. The pulse of depolarization was 50 pA in A (not shown) and 100 pA in C (top trace). A hyperpolarizing pulse of 100 pA was passed in B (top trace). Calibration: 500 pA; 20 mV; 50 msec in A, 100 msec in B, and 500 msec in C.](image-url)

**Table 1. Membrane Properties of Postganglionic Neurons of SD, SHR, and WKY**

<table>
<thead>
<tr>
<th>Neurons</th>
<th>Resting membrane potential (mV)</th>
<th>Input resistance (MΩ)</th>
<th>Spike Amplitude (mV)</th>
<th>Threshold (mV)</th>
<th>Duration (msec)</th>
<th>Hyperpolarizing afterpotential Amplitude (mV)</th>
<th>Duration (msec)</th>
<th>Rheobase (pA)</th>
<th>Chronaxy (msec)</th>
<th>Time constant (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR* (n = 72)</td>
<td>-53 ± 7</td>
<td>83 ± 6</td>
<td>82 ± 2</td>
<td>-36 ± 1.1</td>
<td>6.0 ± 0.3</td>
<td>15 ± 0.6</td>
<td>290 ± 17</td>
<td>135 ± 14</td>
<td>8.1 ± 0.2</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>WKY (n = 63)</td>
<td>-52 ± 1</td>
<td>93 ± 6</td>
<td>81 ± 2</td>
<td>-37 ± 2</td>
<td>4 ± 0.4</td>
<td>13 ± 0.6</td>
<td>310 ± 23</td>
<td>182 ± 17</td>
<td>5.4 ± 0.5</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>SD (n = 40)</td>
<td>-58 ± 1.2</td>
<td>107 ± 9</td>
<td>79 ± 2.2</td>
<td>-43 ± 2</td>
<td>3.5 ± 0.2</td>
<td>14 ± 0.8</td>
<td>300 ± 24</td>
<td>182 ± 18</td>
<td>5.8 ± 0.5</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Analysis of variance</td>
<td>F = 4.95</td>
<td>p = 0.1</td>
<td>F = 0.22</td>
<td>F = 0.3</td>
<td>F = 3.64</td>
<td>F = 1.6</td>
<td>F = 0.27</td>
<td>F = 0.3</td>
<td>F = 3.25</td>
<td>F = 2.5</td>
</tr>
</tbody>
</table>

*Repetitively firing neurons.
†p < 0.01; ‡p < 0.03; §p < 0.05; all other F values were not significant, p > 0.05.
Values represent means ± SEM for the number of determinations shown within parentheses.
TABLE 2. Discharge Properties of Autonomic Neurons of SHR and WKY

<table>
<thead>
<tr>
<th></th>
<th>SHR (72 of 83)</th>
<th>WKY (32 of 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average total no. of spikes per pulse</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Onset firing (Hz)*</td>
<td>29±3.2</td>
<td>22±2</td>
</tr>
<tr>
<td>Maintained firing (Hz)t</td>
<td>11±1</td>
<td>2±1</td>
</tr>
<tr>
<td>No. of cells showing repetitive firing with anodal break</td>
<td>68/72</td>
<td>10/32</td>
</tr>
</tbody>
</table>

*Frequency of action potentials during the first 100 msec of a 400-msec depolarization.
†Frequency of action potentials during the last 300 msec of a 400-msec depolarization.
Values represent means ± SEM of the number of observations indicated within parentheses for a depolarizing current pulse of twice rheobase and 400 msec.

The resting membrane potentials in the neurons of SHR and WKY were consistently depolarized by approximately 5 mV (SHR, -53 ± 0.7 mV, n = 72; WKY, -52 ± 1.0 mV, n = 63) relative to values obtained in normotensive SD (-58 ± 1.2 mV; n = 40). As the absolute values of spike threshold in neurons of SHR and WKY were not different from each other or from those values observed in neurons of SD (Table 1), the mechanism(s) responsible for repetitive spiking in SHR neurons cannot be ascribed to less polarized membrane potentials.

A novel potassium current recently has been described in amphibian and mammalian autonomic neurons. This current is kinetically and pharmacologically unusual. It is maximal between membrane potentials of -40 to -90 mV, and it can be suppressed by muscarine, barium ions, and angiotensin II. The suppression of this current, designated M-current, results in a depolarization of membrane potential, repetitive spiking during and following an intracellular depolarizing current pulse, and anodal-break spiking. Thus it appears that M-currents are directly involved in controlling spike frequency adaptation within sympathetic neurons. We therefore examined the possibility that the loss of spike accommodation observed in the sympathetic neurons of SHR was due to an alteration in the magnitude of M-current by examining the responsiveness of sympathetic neurons of SHR to bath-applied muscarine.

When the superior cervical ganglion neurons from SHR were superfused with (+)muscarine (10 μm), their resting membrane potentials declined by approximately 9 mV (Figure 4; Table 3). Concomitantly, the resting input conductance (G0) decreased by 34% of control values. Between -40 and -90 mV the slopes of the current-voltage curves were decreased in the presence of muscarine. This effect was most prominent...
Membrane Potential (mV)

-109 -89 -89 -79 -99 -59

-200

-300

-400

-500

-600

Figure 4. Current-voltage relationship in the postganglionic neuron of SHR. The curves are determined from the measurement of steady state voltage deflections produced by sequential hyperpolarizing current pulses of from 0 to 500 pA. Closed squares depict normal Locke solution; closed triangles represent the magnitude of the voltage deflections 3 minutes after the addition of (+) muscarine (10 μM). The depolarizing current step in normal Locke solution was 31 pA.

Table 3. Effects of Muscarine on Membrane Potential and Input Conductance of Postganglionic Neurons of SD, SHR, and WKY

<table>
<thead>
<tr>
<th>Neurons</th>
<th>ΔE pm (mV)</th>
<th>ΔG00 (%)</th>
<th>ΔG s (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (n = 6)</td>
<td>+9.2 ± 2</td>
<td>-34 ± 5.5</td>
<td>-44 ± 4.8</td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>+9 ± 1</td>
<td>-18 ± 4.8*</td>
<td>-23 ± 2.1†</td>
</tr>
<tr>
<td>SD (n = 11)</td>
<td>+11 ± 0.8</td>
<td>-38 ± 3</td>
<td>-48 ± 4.4</td>
</tr>
</tbody>
</table>

*Significantly different from SHR and SD by analysis of variance (p < 0.01).
†Significantly different from SHR and SD by analysis of variance (p = 3.8, p < 0.05)

E pm = resting potential (range, -45 to -65 mV for controls); G00 = input conductance (range, 5.4 to 25 ns for all controls); G s = input slope conductance (range, 5.1 to 25 ns for all controls); ΔE = resting potential change produced by muscarine; ΔG00 = % change in input conductance at the predrug resting potential produced by 10 μM (+) muscarine; ΔG s = % change in input slope conductance at the predrug resting potential produced by 10 μM (+) muscarine.

between resting membrane potential and -70 mV (Figure 4). In neurons of SHR, muscarine application produced a 44 ± 5% (n = 6) decrease in the instantaneous slope conductance measured at or near resting potential. These results are similar to those reported by Brown and Constanti21 from normotensive (Wistar) sympathetic neurons. Our experiments with sympathetic neurons of normotensive SD also revealed a similar muscarinic effect; muscarine decreased the instantaneous slope conductance by 48 ± 4.4% (n = 6).

Muscarine treatment of the superior cervical ganglion of SHR also accentuated repetitive spiking during a depolarizing current pulse, specifically increasing the initial frequency by 38% (40 ± 3.3 Hz) and the maintained discharge rate by 64% (18 ± 1.3 Hz; Table 3) without changing membrane properties of these cells other than those expected from a reduced membrane potential. These effects of muscarine on sympathetic neurons of SHR are similar to the actions of this alkaloid on autonomic neurons from Wistar and SD. Thus the results obtained with muscarine indicate that autonomic neurons of SHR possess an apparent normal complement of M-channels.

Effect of Barium on Repetitive Discharge in Neurons

In mammalian autonomic neurons BaCl2 (1-2 mM) elicits repetitive spike discharge to intracellular depolarization and shortens the duration and magnitude of the hyperpolarizing afterpotential.16 In bullfrog sympathetic neurons, bath application of barium mimics the effects produced by muscarine by blocking M-currents.22 To examine further whether altered M-channels could be responsible for repetitive spiking in SHR, we studied the actions of barium on autonomic neurons of SHR. In the range of membrane potentials at which M-current operates, BaCl2 (4 mM) decreased the resting input conductance by 31% and decreased the instantaneous slope conductance by 32% as measured at or near resting potential. These changes in conductance produced by barium were similar, though of smaller magnitude, to those observed in neurons of SHR in the presence of muscarine. Barium, like muscarine, accentuated repetitive spiking in neurons of SHR, which increased the initial discharge rate by 31% (38 ± 3 Hz) and the maintained discharge rate by 40% (15 ± 1.1 Hz; n = 7) and depolarized the membrane potential by 7 mV. These results with barium also support the contention that an alteration in M-currents cannot be a major factor underlying the loss of spike frequency adaptation in sympathetic neurons of SHR.

Calcium Dependence of Repetitive Spiking

The data presented in the previous section indicate that two membrane currents (C- and M-currents) appear normal in autonomic neurons of SHR. We thus examined the nature of the ion(s) responsible for repetitive spikes in neurons of SHR. We added different sodium and calcium antagonists to the perfusion solution. Addition of the calcium antagonists (CoCl2, 5 mM; CdCl2, 0.1-3 mM; or NiCl2, 1 mM) reversibly eliminated repetitive spiking in neurons of SHR and...
REPETITIVE FIRING IN SHR SYMPATHETIC NEURONS/Yarowsky and Weinreich

Figure 5. Calcium dependency of repetitive spikes in two autonomic neurons of SHR. A. The blockade of repetitive spiking in CdCl₂-Locke solution before (1), during the superfusion of 3 mM CdCl₂-Locke solution (2), and after a 15-minute wash in control Locke solution (3). B. The calcium-dependent regenerative discharge in another neuron of the SHR before (1), during the superfusion of 1 μM TTX plus 5 mM TEA (2), and after the addition of 5 mM CoCl₂ to the TTX plus TEA-Locke solution (3). Trace B(3) illustrates the blockade of the calcium-dependent regenerative discharge after the addition of CoCl₂ to the TTX plus TEA superfusate. The resting potential was —44 mV in A(1) and A(3) and —42 mV in A(2). Depolarizing current — top traces of A(1), A(2), and A(3) — was 100 pA in A(1) and A(3) and 150 pA in A(2). In B, resting membrane potential was —50 mV. Depolarizing current (not shown) was 300 pA in B(1) and 400 pA in B(2) and B(3). Calibration: 100 msec, 500 pA and 10 mV in A; 50 msec, 20 and 50 mV in B.

left only a single spike (Figure 5A). The remaining spike could be blocked by the addition of 1 μM TTX to the Locke solution. To demonstrate further that regenerative calcium currents were the charge carriers for the repetitive spiking in autonomic neurons of SHR, we enhanced the maintained firing rate by superfusing the preparation with barium. When the calcium antagonists were then added to the bath, the maintained discharge disappeared and only a single spike remained.

We further examined the possibility that the repetitive spiking in neurons of SHR was generated solely by calcium currents. In these experiments, ganglia were bathed by a Locke solution containing TTX (1 μM) and TEA (5 mM). Under these conditions regenerative sodium currents should be maximally depressed and any contribution by voltage-sensitive calcium currents should be evident.16 The data illustrated in Figure 5 reveal that application of TTX and TEA did not block repetitive spiking. Repetitive spikes in the presence of TTX and TEA were eliminated by the addition of calcium antagonists (Figure 5B, part 3). Thus these results indicate that the inward current of the initial spike is mediated by a predominantly sodium current, whereas the subsequent repetitive spikes are produced by regenerative calcium currents.

Input-Output Relations

Judy et al.3 observed a heightened discharge rate in the cervical sympathetic trunk of the SHR, but they did not determine whether postganglionic discharge also was enhanced. We examined whether there were changes in synaptic efficacy in the ganglia of SHR by constructing input-output curves. The sympathetic trunk was stimulated at 0.25 Hz, a frequency at which there is no synaptic depression or potentiation. These curves, which are derived from extracellular recordings of preganglionic and postganglionic compound action potentials, provide a relation between the amount of transmitter released per presynaptic volley and the intensity of the postsynaptic discharge.25 When the input-output curves of SHR, SD, and WKY were compared, ganglia of both SD and SHR showed similar nonlinear and saturating increases in output as the presynaptic input volley was increased (Figure 6). Input-output curves from the ganglia of WKY revealed a

Figure 6. Input-output relations from the superior cervical ganglia of SD (closed circles), SHR (open circles), and WKY (half-open triangles). Preganglionic and postganglionic compound action potentials were recorded during preganglionic stimulation at 0.25 Hz, and the areas above baseline of the preganglionic and postganglionic responses were measured. Plotted points are the overall means ± sem of the individual outputs for groups of ganglia (SD, n = 12; SHR, n = 8; WKY, n = 8) versus the percentage of the maximal input (see Materials and Methods). The curves are fitted by eye.
different shape. The output increased linearly as the input was increased throughout the entire input range. These input-output curves also indicate that the ganglia of both SHR and SD have similar subliminal fringes and that the subliminal fringe in the ganglia of WKY is greater than in either the ganglia of SHR or SD. None of the output activity recorded in any of the ganglia studied was due to the activation of fibers passing through the ganglia without synapsing because addition of CdCl₂ (0.2 mM) completely blocked the response. These results indicate that the repetitive spiking of the postganglionic neurons of SHR is not directly coupled to a change in transmission through the ganglion, at least for low frequencies (0.25 Hz of presynaptic stimulation).

Discussion

The objective of this investigation was to examine the electrophysiological properties of in vitro autonomic ganglia of the SHR to determine if the sympathetic hyperactivity observed in the intact SHR could be related to an alteration of membrane properties that control postganglionic discharge. We found that postganglionic neurons in the superior cervical ganglion of the SHR have lost the normal accommodative properties of neurons of normotensive strains and show a sustained discharge to intracellular depolarization.

A variety of mechanisms underlying repetitive spiking in neurons could explain the loss of accommodation. Some of the better-characterized mechanisms include (1) the size and duration of the hyperpolarizing afterpotential, (2) the current-voltage characteristics of the cell (i.e., nonlinear rectification), (3) the synaptic connections of the postsynaptic cell (i.e., electrotonic, recurrent collaterals, or interneurons), and (4) the pacemaker potentials. We believe that none of these sources is primarily responsible for the observed repetitive discharge behavior of postganglionic neurons of SHR.

Postganglionic neurons of SHR are not spontaneously active, nor do they show pacemaker potentials. The amplitude and duration of the hyperpolarizing afterpotential following a single action potential are well within the range observed in neurons of normotensive strains (Table 1). Thus neither these biophysical properties nor input resistance, spike amplitude, or spike threshold can explain the differences between the frequency of repetitive spiking observed in the postganglionic neurons of SHR and those from ganglia of WKY or SD. Further, it is unlikely that a synaptic process contributes to the sustained discharge in neurons of SHR, as the repetitive spiking persists in a TTX- and TEA-containing Locke solution.

Although we have no direct evidence concerning the contribution of the K⁺-dependent A-current in repetitive spiking of neurons of SHR, the following arguments would suggest that it does not play a major role. (1) Following a hyperpolarizing current pulse neurons of SHR do not show any abnormal delay in repolarizing to resting potential, which would be indicative of the presence of an enhanced A-current. (2) In neurons of SHR the onset frequency of discharge is greater than the maintained rate (Table 1), which would not be expected in the presence of A-current. (3) In the presence of an A-current blocker, such as 4-aminopyrindine, postganglionic neurons of normotensive SD do not show repetitive discharge to intracellular depolarization.

The sustained neuronal discharge in neurons of SHR is remarkably similar to that observed in normotensive postganglionic superior cervical ganglion neurons superfused with muscarine or angiotensin II. The repetitive discharge evoked by these substances occurs during the passage of depolarizing current and is due to the drug-induced blockade of a unique potassium current designated the M-current. It was because of this similarity that we have previously hypothesized that the unusual repetitive spiking behavior of neurons of SHR could have arisen from an alteration in M-current. The present results obtained with bath-applied muscarine and barium are not supportive of this hypothesis. The percentage change in the slope conductance produced by a standard dose of muscarine (10 μM) or barium (4 mM) is nearly the same in SHR as it was in normotensive superior cervical ganglion neurons. Furthermore, perfusion of the ganglia of SHR with either muscarine or barium potentiated the discharge rate during intracellular depolarization. Thus, quantitatively and qualitatively, our results indicate that M-currents are operational in the postganglionic neurons of SHR. This conclusion is supported by the results obtained by measuring the number of muscarinic binding sites in the ganglion of SHR. As a first approximation the number of muscarinic binding sites might be expected to be correlated with the intensity of M-current. We used the binding of [³H]quinuclidinyl benzilate ([³H]QNB) to determine the number of muscarinic binding sites in the superior cervical ganglion of SHR and genetically related WKY and compared these results with literature values of QNB binding in the superior cervical ganglion of Wistar rats. The numbers of QNB binding sites in ganglia of SHR and WKY were similar both to each other and to published values (unpublished observations of Burt, Weinreich, and Yarowsky).

Although we did not measure the blood pressures of all the animals used in this study, we believe that our findings on the loss of accommodation in postganglionic neurons of SHR are related to the hypertension in this animal. It is generally accepted that adult SHR are hypertensive, though occasionally normotensive SHR and hypertensive WKY are found (Dr. P. Grady, personal communication). In keeping with this notion, our results showed that 12% of neurons of SHR (11 of 83) did not reveal a loss of spike accommodation. These “normal” neurons of SHR were not randomly encountered; rather, they were found in only a few animals (2 of 22), which suggests that these animals may have been normotensive. In three SHR in which both the blood pressure measurements (mean arterial pressure, 170 ± 17 mm Hg) and intracellular recordings from postganglionic neurons were made, these
postganglionic neurons showed a marked loss of accommodation. Similarly, 6% of neurons of WKY (4 of 67 from 2 of 22 animals) showed a pattern of loss of spike accommodation indistinguishable from that observed in neurons of SHR. This finding may reflect the fact that some WKY are hypertensive.

To exclude the effects of genetic differences between the SHR and SD, we recorded a variety of active and passive membrane properties from postganglionic neurons of WKY. The postganglionic neurons of WKY did not show the same form of repetitive discharge to depolarizing stimuli or repetitive calcium spikes (unpublished observation), but they did reveal a tendency for spike doublets or triplets. Other active and passive membrane properties had values similar to neurons of SHR (Table 1). In the presence of muscarine (10 μM) neurons of WKY showed a similar membrane potential change but a smaller percentage change in the slope conductance than did postganglionic cells of SHR (Table 3). At present, we cannot account for the smaller muscarine-induced change in slope conductance in neurons of WKY. Part of the reason for employing superior cervical ganglia of WKY and SD as controls in this study is the continuing debate as to which strain is the adequate control for SHR. Some studies suggest that WKY possess certain latent hypertensive traits.15

Our results suggest that the repetitive postganglionic discharge to intracellular depolarizing current could be related to an enhanced membrane permeability to calcium ions; namely, the observed larger regenerative calcium current. This current was clearly responsible for the sustained action potential discharges observed in neurons of SHR because repetitive spiking persisted in the presence of calcium antagonists such as cadmium, nickel, or cobalt. The prolonged duration of the initial spike of the postganglionic neuron of SHR (Table 1) probably arises from an increased duration of a calcium-dependent “hump” on the falling phase of the spike. The slower rate of rise of the initial spike of SHR and a larger value of chronaxy (Table 1) further substantiate the idea that an enhanced regenerative calcium current exists in the autonomic ganglia of SHR. An enhanced calcium current would be expected to be present at both preganglionic and postganglionic nerve terminals and to be involved in the enhanced evoked release of norepinephrine at peripheral neuroeffector junctions.16 It remains to be resolved whether the change in the calcium current is involved in the maintenance of hypertension in SHR or in its development.

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