Impaired Purinergic Neurotransmission to Mesenteric Arteries in Deoxycorticosterone Acetate-Salt Hypertensive Rats

Stacie L. Demel, James J. Galligan

Abstract—Sympathetic nerves release norepinephrine and ATP onto mesenteric arteries. In deoxycorticosterone acetate (DOCA)-salt hypertensive rats, there is increased arterial sympathetic neurotransmission attributable, in part, to impaired prejunctional regulation of norepinephrine release. Prejunctional regulation purinergic transmission in hypertension is less well understood. We hypothesized that α2-adrenergic receptor dysfunction alters purinergic neurotransmission to arteries in DOCA-salt hypertensive rats. Mesenteric artery preparations were maintained in vitro, and intracellular electrophysiological methods were used to record excitatory junction potentials (EJPs) from smooth muscle cells. EJP amplitude was reduced in smooth muscle cells from DOCA-salt (4 ± 1 mV) compared with control arteries (9 ± 1 mV; P < 0.05). When using short trains of stimulation (0.5 Hz; 5 pulses), the α2-adrenergic receptor antagonist yohimbine (1 μmol/L) potentiated EJPs in control more than in DOCA-salt arteries (180 ± 35% versus 86 ± 7%; P < 0.05). Norepinephrine (0.1 to 3.0 μmol/L), the α2-adrenergic receptor agonist UK 14304 (0.001 to 0.100 μmol/L), the A1 adenosine receptor agonist cyclopentyladenosine (0.3 to 100.0 μmol/L), and the N-type calcium channel blocker ω-conotoxin GVIA (0.0003 to 0.1000 μmol/L) decreased EJP amplitude equally well in control and DOCA-salt arteries. Trains of stimuli (10 Hz) depleted ATP stores more completely, and the latency to EJP recovery was longer in DOCA-salt compared with control arteries. These data indicate that there is reduced purinergic input to mesenteric arteries of DOCA-salt rats because of decreased ATP bioavailability in sympathetic nerves. These data highlight the potential importance of impaired purinergic regulation of arterial tone as a target for drug treatment of hypertension. (Hypertension. 2008;52:322-329.)

Key Words: sympathetic nervous system ▪ excitatory junction potential ▪ vascular neuroeffector junction ▪ ATP ▪ P2X receptors

The nervous system plays an essential role in blood pressure regulation, and sympathetic nerves innervating the splanchnic circulation are particularly important.1 In the periphery, norepinephrine (NE), ATP, and neuropeptide Y are released from postganglionic, sympathetic nerves at the vascular neuroeffector junction to cause vasoconstriction.2 NE mediates a prolonged arterial constriction via an action at α1-adrenergic receptors (ARs). ATP acts at P2X receptors, which are ligand-gated cation channels. ATP causes a rapidly developing but transient depolarization of arterial smooth muscle cell (SMC) membrane potential, called an excitatory junction potential (EJP).3 EJPs that reach the potential at which L-type calcium channels activate cause calcium influx and SMC contraction.4

Increased sympathetic drive to the vasculature contributes to human essential hypertension and animal models of hypertension.5-8 This has been established through measurements of splanchnic NE spillover,9 microneurography,10 and direct amperometric measurements of NE release from sympathetic nerves associated with mesenteric blood vessels.11 NE overflow in response to nerve stimulation is increased in isolated mesenteric vasculature beds of deoxycorticosterone acetate (DOCA)-salt hypertensive rats7,12 indicating that increased adrenergic tone occurs, at least in part, from changes at the neuroeffector junction. However, there are few studies of purinergic transmission in hypertension. The present work focuses on changes in purinergic transmission in mesenteric arteries in the DOCA-salt model of hypertension. DOCA-salt hypertension is characterized by an increase in NE release from sympathetic nerve endings13,14 while renin activity is suppressed.15

Methods

Animals
Sprague-Dawley rats were obtained from Charles River Laboratories (Portage, Mich). Animal use procedures were approved by the institutional animal care and use committee at Michigan State University and were done in accordance with the Guiding Principles

Received January 16, 2008; first decision February 8, 2008; revision accepted June 12, 2008.
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© 2008 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.108.110353

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in the Care and Use of Animals of the American Physiological Society. Rats were acclimated for 2 to 3 days before entry into experimental protocols. Rat chow (Harlan/Teklad 8640 Rodent Diet) and distilled water were provided ad libitum. Rats were housed in temperature- and humidity-controlled rooms with a 12:12-hour light-dark cycle.

DOCA-Salt Hypertension

Male rats (250 to 275 g) were used for all of the studies. DOCA-salt and control rats were prepared, maintained, and assessed according to previously published procedures.14,15

Tissue Preparation

Four weeks after DOCA-salt surgeries, rats were euthanized with an IP injection of sodium pentobarbital (50 mg/kg). The mesentery was surgically removed. Tertiary branches of mesenteric arteries were dissected out, cleaned of adipose and connective tissue, and pinned taut using stainless steel pins (50 μmol/L diameter) in a perfusion chamber coated with Sylgard (Dow Corning). Tissues were superfused with Krebs’ solution of the following composition (mM): NaCl, 117; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 25; NaH2PO4, 1.2; and dextrose, 11. Nifedipine (1 μmol/L; L-type calcium channel antagonist) and prazosin (0.1 μmol/L; α1AR antagonist) were added to the buffer to attenuate vessel constriction during trains of electric nerve stimulation. The buffer was heated to 37°C and bubbled with 95% O2 and 5% CO2. Tissues were allowed to equilibrate for 30 minutes before beginning experiments.

Electrophysiological Recording

Intracellular recordings from individual SMCs were obtained using glass microelectrodes filled with 2 mol/L of KCl (100 to 200 MΩ tip resistance). Impalements were accepted if the following criteria were satisfied: (1) cell penetration was abrupt; (2) membrane potential was at or more than −50 mV; and (3) the membrane potential was stable for ≥5 minutes. Recordings from a single cell lasted 20 to 120 minutes. A Dagan Instruments IX2-700 amplifier was used to record membrane potential. EJPs were evoked using a Krebs’ solution-filled, bipolar, focal stimulating electrode containing 2 parallel Ag/AgCl wire electrodes connected to a Grass Instruments S88 stimulator (Grass Technologies, Astro-Med, Inc). The stimulating electrode was positioned perpendicular to the tissue directly across from the recording electrode. Periartrial nerves were stimulated with a 0.5-millisecond pulse width at the lowest voltage (50 to 120 V), which produced a maximal amplitude EJP. Signals were sampled at 5 kHz and filtered at 500 Hz using an analog-to-digital converter (Digidata 1200, Axon Instruments/Molecular Devices) and Axoscope 9.0 software (Axon Instruments/Molecular Devices). A digital average of 5 sweeps was used to measure the amplitude of EJPs under control and treatment conditions unless otherwise noted. Data were analyzed using Clampfit 9.0 software (Axon Instruments/Molecular Devices).

ATP-Induced Constriction of Mesenteric Arteries

Tissue preparation and the technique used to measure agonist-induced constriction of mesenteric arteries have been described in detail previously.14,15

Drug Application

Drugs were either added to the physiological buffer or applied directly to the recording site using a local drug application system (VC-8 Valve Controller, Warner Instruments). There was an ~1-minute delay between the onset of drug application and drug effect. Tissues were exposed to the drug 5 minutes before testing for drug effects.

Immunohistochemistry

Procedures for preparing mesenteric arteries for immunohistochemical analysis have been published previously.16 After preparation, tissues were preincubated in 0.1 mol/L of PBS (pH 7.2) with triton-X100 (0.5%) for 1 hour and then incubated overnight at 4°C in a dilution (1:200, in triton-PBS) anti-Ca,2.2 (rabbit polyclonal, Alomone Laboratories) and anti-tyrosine hydroxylase (TH; mouse monoclonal, Calbiochem). Next, tissues were washed 3 times in 0.1 mol/L of PBS buffer and then incubated for 1 hour in a dark, humidified chamber at room temperature in diluted fluorescein isothiocyanate–conjugated goat anti-rabbit IgG (1:150) to visualize Ca,2.2 (N-type voltage-dependent calcium channel subunit) staining (Jackson Immunoresearch Laboratories, Inc). Vessels were then washed 3 times with 0.1 mol/L of PBS at 5-minute intervals and coverslipped with Prolong Gold antifade reagent (Molecular Probes [Invitrogen]) for fluorescence microscopy. Specimens were viewed using a Nikon fluorescence microscope (model TE 2000-U), and images were acquired and analyzed using MetaMorph software.

Drugs

Drugs were obtained from Sigma Chemical except for α-Conotoxin GVIA (CTX; Alomone Laboratories). All of the drugs were diluted in deionized water except for prazosin and nifedipine, which were dissolved in 95% ethanol to make a concentrated stock solution. Working solutions of nifedipine and prazosin contained <0.01% ethanol. Final solutions were made in Krebs’ buffer on the day of the experiment.

Statistics

Data are means±SEs, and N values are the number of animals from which the data were obtained. Concentration-response data were fitted using nonlinear regression and the Hill equation (Graphpad Prism). Data were analyzed using a 1-way ANOVA or Student t test for paired or unpaired data, as appropriate. Differences were considered significant when P<0.05.

Results

EJPs in Mesenteric Arteries From Control and DOCA-Salt Rats

Systolic blood pressures of DOCA-salt rats were elevated compared with control rats (196±7 versus 138±2 mm Hg; P<0.001), and body weights were lower (359±14 versus 450±11 g; P<0.001) in DOCA-salt rats compared with control rats. EJPs were blocked by tetrodotoxin (0.3 μmol/L), an Na+ channel blocker, and by PPADS (10 μmol/L), the P2 receptor antagonist, indicating that the EJPs were neurogenic and purinergic (Figure 1A and 1B). The resting membrane potential (RMP) of SMCs from DOCA-salt rats was depolarized compared with control rats (Figure 1C, left; P<0.05), and the average EJP was smaller in DOCA-salt compared with control arteries (Figure 1C, right; P<0.05). The difference in EJP amplitude was not because of differences in RMP, because there was no correlation between EJP amplitude and RMP (Figure 1D). EJP amplitude could be smaller in DOCA-salt arteries because of a decreased reactivity of SMCs to neurally released ATP. We tested this possibility by comparing concentration-response curves for ATP-induced constriction of mesenteric arteries. There were no differences in ATP reactivity in mesenteric arteries from control and DOCA-salt rats (Figure 1E). The pEC50 values were 4.7±0.1 and 4.4±0.1 μmol/L (P>0.05) in control and DOCA-salt arteries, respectively. The maximum constriction was 27±3% and 25±4% (P>0.05) in control versus DOCA-salt, respectively.
Figure 1. Membrane potential and EJPs in mesenteric arteries from control and DOCA-salt rats. A, EJPs are blocked by tetrodotoxin (TTX; 0.3 μmol/L) or PPADS (10 μmol/L). B, Mean data from experiments shown in A (n=4; P<0.05 vs control) indicating that EJPs are neurogenic and mediated by activation of P2X receptors. C, RMP of SMCs in DOCA-salt arteries were depolarized vs those in control tissues (n=22; *P<0.05; vs control). EJPs in arteries from DOCA-salt rats were smaller than those in control arteries (n=22; *P<0.05 vs control). D, EJP amplitude is not correlated with RMP in control (r=0.15; P>0.05) or DOCA-salt arteries (r=0.13; P>0.05). E, ATP concentration-response curves for constriction of mesenteric arteries were similar in control and DOCA-salt arteries.

α2-AR and A1 Adenosine Autoreceptors Regulate ATP Release

Concentration-response curves for agonists at prejunctional α2-AR and A1 adenosine autoreceptors were obtained in tissues from control and DOCA-salt rats (Figure 2). IC50 values and Hill slopes are reported in the Table. NE (0.01 to 3.00 μmol/L) and UK 14304 (0.001 to 0.100 μmol/L), an α2-AR agonist, reduced EJPs equally well in control and DOCA-salt tissues (Figure 2A and 2B). These data suggest that prejunctional α2-ARs couple to inhibition of ATP release from sympathetic nerves, and this effect is not impaired in DOCA-salt hypertension. Similarly, cyclopentyladenosine (0.003 to 0.100 μmol/L), an A1 adenosine autoreceptor agonist, inhibited EJPs equally well in control and DOCA-salt tissues, suggesting that A1 adenosine autoreceptor function is not impaired in DOCA-salt rats (Figure 2C).

N-Type Calcium Channel Function Is not Altered in DOCA-Salt Hypertension

TH is the rate-limiting enzyme in NE synthesis, and TH labeling was used as a marker for sympathetic nerves (Figure 3A). Previous studies showed that N-type calcium channels are involved in transmitter release from parietal sympathetic nerves.17 We used an antibody raised against the α1B calcium channel pore forming subunit, a marker for the N-type calcium channel (Ca2.2; Figure 3B). There was substantial overlap between the nerve fibers containing TH and N-type calcium channel labeling (Figure 3C). There were no differences in the intensity or distribution of labeling for either antigen in tissues from control and DOCA-salt rats. CTX (0.001 to 0.100 μmol/L), an N-type calcium channel blocker, inhibited EJPs equally well in DOCA-salt and control arteries (Figure 3D).

Impaired Facilitation of Purinergic Neuroeffector Transmission in DOCA-Salt Arteries

Synaptic facilitation is a successive increase in the amplitude of a postsynaptic potential during repetitive stimulation. Previous work showed that EJPs recorded from mesenteric arteries facilitate during short trains of stimulation,3 and we investigated the effects of DOCA-salt hypertension on this response. Facilitation was compared in tissues from control and DOCA-salt rats using 5 stimuli at 0.5 Hz with and without yohimbine (1 μmol/L) to ensure that hypertension-associated changes in α2-AR function did not alter facilitation.

Figure 4A shows representative tracings of EJPs elicited by 0.5-Hz trains of nerve stimulation in arteries from control and DOCA-salt treated animals (left: before yohimbine; right: with yohimbine). The ratio of amplitudes of the fifth EJP versus the first EJP in the train was used as a measure of facilitation. In control arteries in the absence of yohimbine, there was little change in EJP amplitude during the stimulus train. However, in the presence of yohimbine, EJP amplitude in control arteries increased during the train (Figure 4A). EJPs recorded from DOCA-salt arteries were smaller than those recorded from control arteries (Figure 4A; lower traces), as discussed above, and little facilitation occurred either in the absence or presence of yohimbine (Figure 4B). In the presence of yohimbine, EJP facilitation in DOCA-salt tissues (86%±7% mV; n=12) was significantly less than in control tissues (180%±35% mV; n=13; P<0.05).

PPADS produced greater EJP inhibition in control compared with DOCA-salt arteries (Figure 4C, left), and this
difference was accentuated in the presence of yohimbine (Figure 4C, right). Because prazosin was present throughout these studies, NE acting at α1ARs is unlikely to contribute to the PPADS-resistant EJP.

Table. Agonists for Prejunctional Autoreceptors at the Sympathetic Neuroeffector Junction

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
<th>pIC50</th>
<th>Hill Slope</th>
<th>N</th>
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</thead>
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<tr>
<td>UK (1 nmol/L to 100 nmol/L)</td>
<td>D</td>
<td>8.2±0.2</td>
<td>−0.8±0.4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8.3±0.3</td>
<td>−1.3±0.5</td>
<td>9</td>
</tr>
<tr>
<td>NE (10 nmol/L to 3 μmol/L)</td>
<td>D</td>
<td>7.2±0.1</td>
<td>−0.9±0.2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.3±0.3</td>
<td>−0.8±0.4</td>
<td>4</td>
</tr>
<tr>
<td>CPA (0.3 nmol/L to 100 nmol/L)</td>
<td>D</td>
<td>8.3±0.2</td>
<td>−1.0±0.2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8.3±0.2</td>
<td>−1.0±0.2</td>
<td>5</td>
</tr>
</tbody>
</table>

Analysis of the concentration response curves for NE, UK 14304, and CPA on EJP amplitude in control and DOCA-salt arteries. Data were obtained from the indicated number of animals and are means±SEs. D indicates DOCA-salt; C, control; CPA, cyclopentyladensosine; UK, UK14304.

EJP Rundown in Arteries From DOCA-Salt Rats

High-frequency trains of nerve stimulation can be used to deplete stores of neurotransmitters. The rate of decline in the amplitude of the postsynaptic or postjunctional response can be used as a measure of the amount of transmitter stored in nerve terminals. The reduced amplitude of the EJP recorded from DOCA-salt arteries could be because of reduced nerve terminal stores of ATP in DOCA-salt hypertension. Therefore, we measured EJP amplitudes during 10-second trains of 10-Hz stimulation. Because we used a high frequency of stimulation, EJPs summated to produce a sustained depolarization until ATP stores were depleted from the sympathetic nerve endings. In control arteries, EJPs occurred throughout the stimulus train leading to sustained depolarization (Figure 5A). In DOCA-salt arteries, EJP amplitude declined rapidly, leading to only a transient depolarization (Figure 5B). EJP rundown was compared between control and DOCA-salt arteries by calculating the average amplitude of the first 5 EJPs and the 10th, 20th, and 30th EJPs (Figure 5C). These data show reduced facilitation in DOCA-salt arteries and also that EJP rundown was greater in DOCA-salt compared with control arteries (Figure 5B). Rundown was quantitated by dividing the amplitude of the peak EJP by the amplitude of the 30th EJP, and this analysis confirmed that rundown was greater in the DOCA-salt compared with control tissues (Figure 5D; P<0.05).

Impaired EJP Recovery After Rundown in DOCA-Salt Arteries

Increased rundown of EJPs in DOCA-salt arteries suggests a decreased availability of vesicular ATP in sympathetic nerve terminals. This may be because of impaired synaptic vesicular filling with ATP. To test this hypothesis, the time to recovery of EJP amplitude was assessed after rundown. Restoration of EJP amplitude after rundown was used as an indication of the efficiency of vesicular refilling with ATP. After stimulation at 10 Hz for 10 seconds, a single EJP was elicited after latencies of between 0.2 and 2.0 seconds (Figure 6A). A ratio of the initial EJP amplitude in the train to the recovery EJP amplitude was calculated as a recovery index at each recovery time. At 0.2 seconds, EJP amplitude in control arteries was not different from the initial EJP in the stimulus train, and EJP amplitudes at 1.0 and 2.0 seconds were significantly greater than the initial EJP (Figure 6B). EJP amplitude in DOCA-salt arteries did not fully recover until 2 seconds after the end of the stimulus train. The recovery ratio was significantly different between control and DOCA-salt arteries at 0.5, 1.0, and 2.0 seconds (Figure 6B).

Discussion

NE and ATP are cotransmitters released from sympathetic nerves supplying arteries. Previous studies measured higher concentrations of NE and ATP in overflow solutions from DOCA-salt compared with control arteries when long trains of stimulation were used to evoke transmitter release from sympathetic nerves. Increased sympathetic nerve activity, increased NE release, and increased neurogenic vasoconstriction all occur in hypertension. There have been few studies of hypertension-associated changes in the puri-
ergic component of sympathetic neuroeffector transmission in arteries. ATP acts at P2X receptors on SMCs to cause EJPs of which the amplitude can be an indirect measure of ATP in the neuroeffector junction. Although this measurement is not quantitative, it does measure ATP release from nerves near the sites of action. ATP in overflow solutions would likely come from neural and nonneural sources. Our studies found a decrease in EJP amplitude in mesenteric arteries from DOCA-salt rats. This is a novel finding. Previously, EJPs were measured in mesenteric arteries of spontaneously hypertensive rats, a genetic model of hypertension, where it was found that the average EJP amplitude was increased. Another study reported similar results in the prostatic portion of vas deferens in spontaneously hypertensive rats. The authors concluded that increased postjunctional responsiveness to nerve-released ATP was responsible for increased EJP amplitude. However, this and previous studies showed that there is no difference in the concentration-response curve for ATP-induced constriction of control and DOCA-salt arteries, suggesting that postjunctional reactivity to ATP is not altered in DOCA-salt hypertension. We found that the RMP of SMCs in DOCA-salt arteries was depolarized compared with control arteries. The depolarized membrane potential could reduce EJP amplitude by reducing the driving force for cation movement through the P2X receptor. However, we also found that EJP amplitude was unrelated to membrane potential in either sham or DOCA-salt arteries. These data suggest that postjunctional changes are not the primary cause of decreased EJP amplitude in DOCA-salt arteries. Decreased EJP amplitude in DOCA-salt hypertension but increased EJPs in spontaneously hypertensive rats can be explained by differences in the hypertension models. Angiotensin acting at angiotensin II type 1 receptors increases EJPs in rat mesenteric arteries. The renin-angiotensin system is activated in spontaneously hypertensive rats, whereas the renin-angiotensin system is suppressed in DOCA-salt hypertension, and this could account for the difference in EJP amplitude in the 2 hypertension models.

We also found that EJPs recorded from DOCA-salt arteries were less sensitive to inhibition by PPADS than EJPs in control arteries and that this difference was accentuated by yohimbine. These data suggest that additional mediators or receptors contribute to the EJP in DOCA-salt arteries. The time course of EJPs did not obviously change in DOCA-salt hypertension, so it is likely that ligand-gated ion channels similar to P2X receptors mediate the EJP in DOCA-salt arteries. P2X4 and P2X6 receptors are PPADS insensitive. Perhaps P2X4 or P2X6 receptor upregulation occurs in DOCA-salt arteries, and this would account for reduced EJP sensitivity to PPADS. Additional studies are required to resolve this issue.

Prejunctional autoreceptors regulate transmitter release at the neuroeffector junction. Prejunctional α2ARs are activated by nerve-released NE to negatively regulate both NE and ATP release. Sympathetic nerves release ATP, along with ecto-ATPases and 5'-nucleotidases, which quickly degrade ATP into ADP, AMP, and adenosine. Adenosine acts at prejunctional A1 adenosine receptors, which also negatively regulate NE and ATP release. Importantly, impaired prejunctional α2AR function is associated with hypertension in humans and in animal models of hypertension, including DOCA-salt hypertension. Because the α2AR also regulates ATP release, we tested the hypothesis that the α2AR may also be involved in altered purinergic neurotransmission. However, UK 14304 and exogenous NE inhibited EJPs equally well in DOCA-salt and control arteries. Although previous studies have shown that the α2AR coupling to NE release is impaired in DOCA-salt hypertension, our data are the first to show that α2AR coupling to the regulation of ATP release caused by a single stimulus is maintained in DOCA-salt hypertension. These data correspond with previ-
ous reports that the $\alpha_2$-AR is more tightly coupled to NE compared with ATP release.32 We also found that the A1R agonist cyclopentyladenosine inhibited EJPs equally well in control and DOCA-salt arteries, indicating that A1R coupling to ATP release caused by a single stimulus is maintained in DOCA-salt hypertension.

Because direct inhibition of nerve terminal calcium channels is one way in which autoreceptors decrease neurotransmitter release, we investigated the function of prejunctional calcium channels. Immunohistochemical studies demonstrated that immunoreactivity for N-type calcium channels colocalized with TH immunoreactivity in periarterial nerve fibers, suggesting that sympathetic nerves express N-type calcium channels.33–35 Therefore, we used $\omega$-CTX GVIA35 to determine whether the sensitivity of purinergic transmission to N-type calcium channel blockade is altered in DOCA-salt hypertension. EJPs in control and DOCA-salt arteries were equally sensitive to $\omega$-CTX GVIA so that N-type calcium channel coupling to ATP release was not altered DOCA-salt hypertension. Together, these data suggest that alterations in the $\alpha_2$-AR, A1R, or N-type calcium channels are not responsible for impaired purinergic neurotransmission in DOCA-salt hypertension.

**Decreased ATP Bioavailability in DOCA-Salt Hypertension**

We next tested the hypotheses that decreased ATP availability in the nerve terminal was responsible for decreased purinergic neurotransmission in DOCA-salt hypertension. If there was decreased ATP availability in sympathetic nerves from DOCA-salt rats, then high-frequency stimulation would lead to more rapid rundown of EJP amplitude in DOCA-salt compared with control arteries. We found that rundown was more rapid and complete in arteries from DOCA-salt rats compared with control rats. There are several possible explanations for these data. First, there could be a decrease in nerve terminal content of ATP in DOCA-salt tissues. Mitochondrial damage is both a result of and a contributing factor to oxidative stress known to occur in hypertension.36 Although mitochondrial damage in itself would directly result in decreased ATP production, increased ROS may also reduce vesicular ATP stores. ROS alter neurotransmission in animal models of diabetes mellitus. For example, ROS impair sympathetic neurotransmission to the vas deferens of streptozotocin-diabetic rats.37 Another possibility for decreased purinergic neurotransmission is increased ATP degradation because of an increase in metabolic demands of sympathetic nerves in hyper-
tensive rats. Sympathetic nerve activity is increased in DOCA-salt rats, and this would result in increased nerve terminal Ca\(^{2+}\)/H\(_{11001}\) concentrations, vesicular recycling, and neurotransmitter release, all of which are ATP dependent. Thus, ATP stores could quickly be depleted in the nerve endings in hypertension.

Second, decreased purinergic neurotransmission could be a result of increased NE release. Previous studies have shown interplay between the regulation of ATP and NE stores. Because NE release is increased in hypertension, this could inhibit EJPs via activation of prejunctional \(\alpha_2\)AR. However, we showed that blocking the prejunctional \(\alpha_2\)AR with yohimbine did not restore EJP amplitude. Therefore, increased NE release is most likely not the cause for decreased EJP amplitude in DOCA-salt arteries.

Finally, we tested the possibility that vesicular packaging of ATP is compromised in DOCA-salt hypertension, because the ability for refilling would be compromised with an overall decrease in ATP bioavailability in the nerve terminals. To examine vesicular refilling, we analyzed time to recovery after rundown. The latency to recovery was longer in DOCA-salt hypertensive arteries. This indicates that impaired vesicular refilling with ATP after depletion is a potential mechanism for decreased purinergic neurotransmission in DOCA-salt hypertension.

**Perspectives**

Decreased ATP availability in periarterial sympathetic nerves has several implications for the pathogenesis of hypertension. Decreased ATP release, combined with increased NE release, suggests a phenotypic switch from NE to ATP as the primary mediator of mesenteric arterial constriction in DOCA-salt hypertension. Different pathways mediate the postjunctional effects of ATP and NE in mesenteric arteries. Constrictions caused by ATP are transient because of rapid desensitization of P2X\(_1\) receptors and degradation of ATP by nucleotidases. However, NE would produce prolonged constrictions via activation of \(\alpha_1\)-ARs, which desensitize slowly and which couple to a cascade of signaling events, resulting in sustained calcium elevations and

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**Figure 5.** EJP rundown in arteries from DOCA-salt and control rats. A, EJPs elicited by nerve stimulation for 5 seconds are shown for arteries from control (left) and DOCA-salt (right) rats. At this stimulation frequency, individual EJPs summate to cause a sustained depolarization in control arteries. B, EJP rundown in arteries from DOCA-salt rats and the sustained depolarization declines to baseline during the stimulus train. C, Mean data from experiments similar to those shown in A and B showing complete rundown in DOCA-salt but not control arteries. D, Comparison of EJP rundown in control and DOCA-salt arteries. Rundown was quantified as the ratio of the amplitude of the 30th EJP vs the peak EJP (sixth EJP) in the train. Rundown was greater in DOCA-salt arteries (*\(P<0.05\); DOCA-salt vs control).

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**Figure 6.** Impaired EJP recovery after rundown in arteries from DOCA-salt rats. A, Representative recording from a control artery showing complete recovery of EJP amplitude within 1 second of the end of the stimulus train. B, Summary data from studies done in control and DOCA-salt arteries. In control arteries, EJP amplitude at 0.2 seconds was not different from the initial EJP amplitude in the train. At 1 and 2 seconds after the end of the train, the EJP amplitudes were larger than the initial EJP (*\(P<0.05\) vs initial amplitude). In DOCA-salt arteries, the EJP at 0.2 seconds was smaller than the initial EJP (*\(P<0.05\)). EJP amplitudes in DOCA-salt arteries were smaller than in control arteries at 0.5, 1.0, and 2.0 seconds (&\(P<0.05\); DOCA-salt vs control).
phosphorylation of contractile proteins. As the mesenteric bed contributes to total peripheral resistance, sustained arterial constrictions would contribute to increased vascular resistance known to occur in hypertension.

Sources of Funding
This work was supported by a National Institutes of Health grant (HL070687). S.L.D. is a recipient of an American Heart Association predoctoral fellowship (0615576Z).

Disclosures
None.

References
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_Hypertension_. 2008;52:322-329; originally published online July 7, 2008;
doi: 10.1161/HYPERTENSIONAHA.108.110353

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/52/2/322

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