Augmented Sympathetic Nerve Activity and Pressor Responsiveness in DOCA Hypertensive Rats

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SUMMARY Spike potentials in abdominal sympathetic nerves were recorded together with aortic blood pressure before and during electrical stimulation of the posterior hypothalamus in urethane-anesthetized rats. Both the initial rate of sympathetic nerve firing and the subsequent acceleration produced by hypothalamic stimulation were higher in deoxycorticosterone acetate (DOCA) hypertensive than in normotensive rats. Pressor responsiveness was generally augmented, but responses to hypothalamic stimulation were increased far more than those to injected norepinephrine. Vasodepression produced by blocking autonomic ganglia pharmacologically with pentolinium was also more pronounced in DOCA hypertensive rats than in normotensive controls. These results support the conclusion that a centrally induced sympathetic hyperactivity is important for maintaining the blood pressure elevation in rats with established DOCA hypertension.

KEY WORDS • blood pressure • DOCA hypertension • ganglion blockade • hypothalamus • norepinephrine • sympathetic nerves

TWO conflicting but almost equally popular views now exist on whether sympathetic hyperactivity participates in causing deoxycorticosterone acetate (DOCA) hypertension. Supporting the belief that sympathetic hyperactivity is essential, the hypotension induced by combining adrenalectomy with chemical sympathectomy has been shown to result in identical blood pressure levels in adult normotensive and DOCA hypertensive rats.1 Also, to restore blood pressure levels following splanchnicotomy during pentobarbital anesthesia, the splanchnic nerves had to be stimulated at much higher frequencies in DOCA hypertensive than in other rats.2 Furthermore, destruction of central catecholaminergic neurons through intracerebroventricular injection of 6-hydroxydopamine prevents not only the elevation in tail-cuff systolic pressure3 but also the attendant increase in plasma norepinephrine4 in unanesthetized DOCA hypertensive rats. On the contrary, however, others have found DOCA hypertension either unaffected in adult rats5-9 or made even worse in neonates9-10 after chemical sympathectomy. Observations that seem contradictory have also been made in our laboratory: DOCA hypertensive rats have an enhanced pressor responsiveness to hypothalamic stimulation,10 yet their pressures fell only to the same levels as those of normotensive rats when the hypothalamus was destroyed.11 In an attempt to find a logical explanation for this, we recorded sympathetic nerve activity directly before and during hypothalamic stimulation. To determine if the sympathetic overactivity thereby revealed would account for the elevation in blood pressure, we also measured vasodepression occurring after pentolinium-induced ganglion blockade.

Methods

Female Sprague-Dawley rats, 3 weeks old, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Soon after arrival, all were anesthetized with sodium amobarbital (8 mg/100 g i.p.) and their left kidneys removed. Silicon rubber molds containing DOCA (200 mg/kg) were implanted subcutaneously in eight rats, according to the method of Ormsbee and Ryan,12 while molds of silicone rubber alone were similarly implanted in 10 others. Thereafter, the rats were given 0.9% sodium chloride solution instead of water for drinking, and systolic pressures were measured weekly using a tail-cuff method validated for use in awake rats.13 Two months later, systolic pressures (mm Hg ± SEM) averaged 119 ± 4 in sham-operated, and 185 ± 9 in DOCA hypertensive rats (p < 0.001); cor-
responding averages for body weight (232 ± 7 and 236 ± 13 g respectively) or heart rate (371 ± 7 and 352 ± 12/min respectively) were not significantly different. All rats were then reanesthetized with amobarbital, and a concentric electrode (NE-100 with chronic connectors, Rhodes Medical Instruments, Woodland Hills, CA) was placed in the posterior hypothalamus at stereotaxic coordinates antero-posterior 4.6, lateral 1.0, and dorsoventral −2.5. Electrodes were fixed to the skull with stainless steel screws and dental cement. Following electrode implantation, rats were individually caged in an air-conditioned room, fed a standard laboratory chow ad libitum, and allowed to recover for at least 1 week before experimentation.

During experiments, rats were anesthetized with urethane (0.1 g/100 g i.p.), and catheters were inserted into a jugular vein for drug injection and into the lower abdominal aorta for blood pressure recording. The abdominal plexus was exposed, and, with the aid of a stereoscopic microscope, a bipolar stainless-steel electrode ( uninsulated tips 1 mm apart) was placed on the major nerve bundle accompanying the superior mesenteric artery immediately below the celiac ganglion (hereafter referred to as the "abdominal sympathetic nerve"). Nerves and electrode tips were immersed in mineral oil to reduce tissue-drying. Spike potentials were amplified (Grass P15 AC amplifier) and monitored on a storage oscilloscope (Tektronix 5111). To reduce noise during these recordings, spontaneous respiration was abolished by paralyzing skeletal muscles with decamethonium bromide (Syncurine, 0.2 mg/100 g i.v.) and connecting the rats to a respirator ventilated with a mixture of 50% oxygen and 50% nitrogen.

Analog signals for aortic pressure and nerve activity were recorded continuously on magnetic tape. To quantify nerve activity, original analog signals were played back from tape into an ink-writing recorder (F. Haer and Co., Brunswick, ME) to convert individual spikes into uniform pulses and delete background noise. Because residual activity remaining after ganglion blockade with pentolinium was the same as that after crushing the nerve, the low-level control of the window discriminator was routinely set to filter background noise persisting after pentolinium injection. The number of individual pulses per second were counted with a rate analyzer (F. Haer and Co.) whose output was recorded separately as a histogram, digitized by a computer interface, and then printed by a programmed calculator (Monroe 1860) for rates under 100 spikes/sec (higher rates were counted manually from the histogram).

Blood pressure was recorded continuously by connecting the aortic catheter through Tygon tubing to a small-volume-displacement pressure transducer (Statham P23Gb). A square-wave stimulator (Grass S-48 with constant current unit) was used to deliver 50, 100, and 200 μA currents (pulse duration 1 msec, frequency 100/sec) in 10-second trains to the posterior hypothalamus. Two drugs were routinely injected through the jugular vein catheter: pentolinium tartrate (Ansolyn), 0.5 mg (salt)/100 g, and norepinephrine bitartrate (Levophed), 100, 200, and 400 ng (base)/100 g.

At the end of each experiment, a 2 mA direct current was passed through the hypothalamic electrode for 5 seconds to produce a small lesion and thereby facilitate histologic localization of the electrode tip. Then, 10% formalin was perfused into the brain through a needle inserted into the ascending aorta via the left ventricle. Excised brains were stored in formalin until sectioning; transverse sections 40 μ thick were examined through a stereomicroscope, and electrode tip locations verified by comparison with the atlas by Pellegrino and Cushman. All lesion sites were immediately lateral to the fornix and mamillothalamic tract; in addition to the posterior hypothalamic nucleus, other structures within or adjacent to the terminal lesion included the lateral hypothalamic area, premamillary nuclei, zona incerta, and median forebrain bundle.

Data (averages ± SEM) from both rat groups were analyzed using t tests for comparing means of independent samples, and differences at a 5% level (p < 0.05) were considered significant.

Results

Initial baselines for both blood pressure and sympathetic nerve activity were elevated in DOCA hypertensive rats. Like the difference shown previously with the tail-cuff method when these rats were awake, mean aortic pressure (mm Hg ± SEM), which averaged 140 ± 7 in DOCA hypertensive rats, was higher (p < 0.001) during urethane anesthesia than the corresponding average of 112 ± 2 mm Hg in sham-operated controls. Similarly, the frequency of sympathetic nerve firing (spikes/3 sec ± SEM), which averaged 74 ± 9 in DOCA hypertensive rats, was significantly higher (p < 0.05) than the corresponding average of 52 ± 5 spikes/3 sec in sham-operated ones. By contrast, the averages for heart rate/min ± SEM of stimulation on sympathetic nerve firing were always sham-operated rats were not significantly different.

Accelerated Nerve Firing During Hypothalamic Stimulation

Electrical stimulation of the posterior hypothalamus increased nerve firing rates almost instantly in all rats. Accelerated rates persisted throughout the 10-second period of stimulation, but their magnitude was usually greatest during the first 3 seconds and then diminished spontaneously thereafter (though still staying well above prestimulation levels) despite continued stimulation. In both rat groups, the amount of acceleration was proportional to the current strength (within the range tested) applied to the hypothalamus; the acceleration occurring during the first 3 seconds of stimulation with any current was invariably more pronounced in DOCA hypertensive than in sham-operated rats (table 1).
Comparison of Responses to Hypothalamic Stimulation and to Injected Norepinephrine

As in previous studies, the effects of hypothalamic stimulation on sympathetic nerve firing were always accompanied by measurable increases in aortic pressure, which were also much more prominent in DOCA hypertensive than in sham-operated rats (fig. 1). Aside from the pressor effects of hypothalamic stimulation, similar responses induced by injecting graded doses of norepinephrine were also compared. Both stimuli consistently evoked measurable increases in mean aortic pressure, which were larger in DOCA hypertensive rats.

<table>
<thead>
<tr>
<th>Current strength (µA)</th>
<th>Stimulus duration (sec)</th>
<th>Rat group*</th>
<th>p value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sham-operated (spikes/3 sec)</td>
<td>DOCA hypertensive (spikes/3 sec)</td>
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<tr>
<td>50</td>
<td>3</td>
<td>81 ± 12</td>
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*Results are presented as increases in absolute firing rates over base line levels obtained in 10 sham-operated and eight DOCA hypertensive rats.

n.s. = not significant.

FIGURE 1. Pressor and neural responses to hypothalamic stimulation in a sham-operated (A) and a DOCA hypertensive (B) rat. Upper panels: Tracings of phasic aortic pressure (mm Hg; note scale change between panels). Middle panels: Original nerve spike potentials. Lower panels: Integrated nerve activity (spikes/sec). The arrow marks the start of stimulation for 10 seconds at the current strength (µA) indicated by the large number.
hypertensive than in sham-operated rats (table 2). In general, pressor responses to hypothalamic stimulation tended to be more strongly enhanced than those to injected norepinephrine, but these differences in magnitude of enhancement were small. For instance, in sham-operated rats, hypothalamic stimulation with 100 μA produced pressor responses averaging 15 ± 2, which are almost the same as the average of 14 ± 1 elicited by 200 ng/100 g of norepinephrine. By contrast, in DOCA hypertensive rats, the same stimulus strengths produced average pressor responses of 40 ± 4 and 31 ± 4 respectively. With either stimulus, heart rates were almost invariably reduced, but none of the differences between rat groups was significant.

**Vasodepression Caused by Ganglion Blockade**

As ganglioplegia was induced with pentolinium routinely at the end of each experiment, the ensuing fall in aortic pressure provided a readily available means of estimating the amount of pre-existing sympathoadrenal activity. The mean aortic pressure was reduced more \( (p < 0.005) \) in DOCA hypertensive \((-78 ± 6 \text{ mm Hg})\) than in sham-operated \((-53 ± 3 \text{ mm Hg})\) rats; consequently, baseline pressures after ganglion blockade were almost equal in both groups \((65 ± 5 \text{ and } 61 ± 2 \text{ mm Hg respectively})\).

**Discussion**

Much of the evidence now being disputed about the role of sympathetic hyperactivity in DOCA hypertension comes from experiments in which the presence or absence of sympathetic overactivity was inferred indirectly from blood pressure changes produced by either immuno- or chemical sympathectomy. Immunosympathectomy completely prevented development of hypertension in one study, but was found ineffective in another published in the same year. Similarly, contradictory results have been reported following chemical sympathectomy produced with 6-hydroxydopamine. Whereas two reports claimed that this drug was successful in protecting against subsequent elevation of blood pressure, many other attempts failed, possibly because sympathectomies were incomplete. Adrenergic nerve endings in blood vessels regenerate more rapidly, and are less readily depleted of their catecholamine content following chemical or immunosympathectomy.

Nevertheless, even more perplexing results were recently reported by Provoost and de Jong, who found that the development of DOCA hypertension was potentiated, rather than inhibited, in rats subjected to neonatal sympathectomy. Destruction of sympathetic nerves was presumed to be functionally complete since pressor responses could not be elicited with either tyramine or electrical stimulation of various brain or spinal centers. The antihypertensive protection previously obtained by others was attributed to hypotensive sensitization by anesthesia, but why this occurred only in DOCA hypertensive rats was left unexplained.

Additional evidence attesting to the existence of sympathetic hyperactivity in rats with established DOCA hypertension can be obtained from our results. Despite wide variations from rat to rat in the frequency of spontaneous nerve firing, baselines for sympathetic nerve activity were significantly faster at the onset in DOCA hypertensive than in normotensive rats. And when the posterior hypothalamus was subsequently stimulated with graded current strengths, the magnitude of both the added acceleration in nerve firing (table 1) and the ensuing pressor responses (table 2) were invariably stronger in DOCA hypertensive rats than in normotensive ones.

It seems logical to assume that this enhancement of pressor responsiveness was due mainly to the accompanying increase in sympathetic nerve discharge, but this cannot be the sole mechanism involved because pressor responses to injected norepinephrine were also augmented, thereby implying that vascular sensitivity had also increased. Exactly how much each mechanism contributed is difficult to determine. For instance, upon hypothalamic stimulation with 50 μA, the increase in sympathetic nerve firing was only about two times larger in DOCA hypertensive rats, but the corresponding increment in pressor response was almost four times larger. Since pressor responsiveness to injected norepinephrine was only doubled, the discrepancy could conceivably indicate an enhanced release of endogenous norepinephrine. Because differences in the magnitude of both the sympathetic nerve firing and pressor responses became smaller as the strength of the hypothalamic stimulation intensified, it seems likely that the extent of the neural contribution to the enhanced pressor responsiveness diminished as current strengths for stimulation were increased. Finally, still another sign of sympathoadrenal hyperactivity is provided by the greater vasodepression resulting in DOCA hypertensive rats following pharmacologic blockade of autonomic ganglia with pentolinium.

If sympathetic overactivity really accounts for most of the blood pressure elevation once DOCA hypertension becomes established, then interruption of the
siderent nerve pathway at or below the site of overactivity should lower the blood pressure more markedly in DOCA hypertensive rats than in others. Of the procedures tested thus far (other than immunoo or chemical sympathectomy), a greater hypotensive effect did occur following pithing or section of the spinal cord, or after pharmacologic ganglion blockade. Aside from pentolinium, two other ganglion-blocking drugs, namely, chlorisondamine and mecamylamine, have also been tried but interpretation of the results is complicated by the fact that, regardless of the site of action, other hypotensive drugs such as furosemide, hydrochlorothiazide, or diazoxide, have all been found consistently more effective in DOCA hypertensive rats than in normotensive controls. Another drug with selective but weak antihypertensive effects that could be centrally mediated is propranolol.

Because identical blood pressure levels can be produced in normotensive and DOCA hypertensive rats by cutting the spinal cord at the C6–C7 level, but not by destroying the posterior hypothalamus, sympathetic nerve hyperactivity may be occurring at sites on the neuraxis lower than the posterior hypothalamus. Thus, the apparent contradiction between enhanced pressor responsiveness to hypothalamic stimulation on the one hand, and the lack of a greater hypotensive effect upon hypothalamic destruction on the other, would be resolved. In line with this, and based on the selective decrease of norepinephrine turnover in the brain stem of DOCA hypertensive rats, Chalmers proposed that increased sympathetic nerve activity at the periphery results from reduced inhibition by medullary noradrenergic neurons terminating at the vasomotor center.

References

Augmented sympathetic nerve activity and pressor responsiveness in DOCA hypertensive rats.
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