Chemical Sympathectomy Alters the Development of Hypertension in Miniature Swine

Gail D. Thomas, Kathleen P. O'Hagan, and Edward J. Zambraski

To determine if the neurotoxin 6-hydroxydopamine could be used to chemically sympathectomize neonatal miniature swine, eight newborn swine were treated with 6-hydroxydopamine beginning on the first day after birth and continuing at regular intervals for the next 6 months. Six littermates served as controls and received vehicle injections. A significant reduction in the pressor response to intravenous tyramine (95%) and in the tissue norepinephrine content of the kidneys, left ventricle, and gastrocnemius muscle (more than 93%) provided evidence for an effective long-term sympathectomy in the 6-hydroxydopamine-treated animals. In addition, the blood pressure response of these young, chemically sympathectomized swine to chronic deoxycorticosterone acetate treatment was evaluated. Mean arterial pressure before deoxycorticosterone was similar in the 6-hydroxydopamine-treated (116±2 mm Hg) and control (125±5 mm Hg) groups. One week after deoxycorticosterone, mean arterial pressure had risen significantly by 20-22 mm Hg in both groups. Blood pressure continued to increase in the control group, reaching a value of 163±6 mm Hg by the third week after treatment. In contrast, mean arterial pressure in the 6-hydroxydopamine group did not increase further during weeks 2 and 3 after deoxycorticosterone. In conclusion, chronic treatment of neonatal swine with 6-hydroxydopamine produced an animal model with an effective, general, peripheral sympathectomy. The significant attenuation of the hypertensive response in these sympathectomized animals lends further support to the hypothesis that an intact sympathetic nervous system is necessary for the full expression of deoxycorticosterone hypertension in miniature swine. (Hypertension 1991;17:357-362)

A generalized peripheral sympathectomy has been achieved by administration of the neurotoxin 6-hydroxydopamine (6-OHDA) in a variety of species, including rats, rabbits, dogs, cats, and sheep.1-5 In adult animals, 6-OHDA specifically destroys adrenergic nerve terminals leaving the cell bodies intact, whereas in neonates, destruction of the entire neuron occurs.6 A transient sympathectomy results in adult animals, as evidenced by the rapid regrowth of nerve terminals within several weeks and complete functional recovery within several months.1 However, in neonates the sympathectomy appears to be more complete and more permanent than it is in adults.6

Increasing use of the miniature swine as a model for biomedical studies and work from this laboratory focusing on sympathetic control of renal function led us to become interested in the possibility of using 6-OHDA to chemically sympathectomize swine. Validation of such a model would obviate the technical difficulties associated with surgical denervation. Chronic surgical denervation of specific organs or tissues is difficult to perform and is limited in effectiveness due to a rapid reinnervation and recovery of sympathetic function after denervation. Also, the completeness of surgical denervation usually cannot be fully verified until the end of the study by the analysis of tissue norepinephrine. Therefore, one of our objectives in this study was to determine the efficacy and feasibility of using 6-OHDA in neonatal swine to obtain an effective, long-lasting, peripheral sympathectomy.

Studies from this laboratory have characterized the deoxycorticosterone acetate (DOCA)-treated miniature swine as a hypertensive animal model that...
exhibits elevated sympathetic nervous system activity, elevated total peripheral resistance, low plasma renin activity (PRA), and abnormal renal function. We have previously shown that the onset of DOCA hypertension is delayed by prior surgical renal denervation and that renal denervation in swine with hypertension10 and that renal denervation in swine with established DOCA hypertension normalizes blood pressure without a concomitant increase in sodium excretion.11 To further study the role of the sympathetic nervous system in the development of DOCA hypertension, we chose to evaluate the blood pressure response to DOCA administration of 4-5-month-old swine that were chronically treated from birth with 6-OHDA.

Methods

Fourteen male and female miniature swine were used for this study (Deerfield Mini-Pigs, Deerfield, N.J.). Eight of these animals were treated with 6-OHDA as described below. The remaining six animals served as controls and received vehicle injections. All of the piglets were housed together with their mother until they were weaned at approximately 10-12 weeks of age. At that time, the pigs were separated and housed individually in pens. All animals were fed a daily swine ration and permitted water ad libitum.

On days 1, 2, and 3 after birth, eight of the animals were given intraperitoneal injections of 25, 50, and 75 mg/kg of 6-OHDA hydrobromide (Aldrich, Milwaukee, Wis.), respectively. The drug was dissolved in 0.9% saline containing 1 mg/ml ascorbic acid and was made immediately before injection. During the second and third weeks after birth, the animals were treated with 6-OHDA (75 mg/kg i.p.) three times a week. To insure that the animals remained sympathectomized, weekly injections of 6-OHDA (75 mg/kg i.p.) were given from week 4 until completion of the study approximately 5 months later. The injection volume never exceeded 5 ml.

Experimental Protocol

Under halothane anesthesia and sterile conditions, tygon catheters were placed in the left common carotid artery and left external jugular vein of each animal at 17-20 weeks of age. The catheters were exteriorized on the dorsal surface of the neck. After several days of recovery, the animals were placed in a hammock sling, with which they had previously been familiarized, for the measurement of conscious mean arterial pressure (MAP). A Statham pressure transducer (Statham Division, Gould, Inc., Oxnard, Calif.) was connected to the arterial catheter to measure MAP. Pulsatile and electronically averaged MAP were recorded on a direct writing recorder (Grass Instrument Co., Quincy, Mass.). Heart rate (HR) was determined from pulsatile blood pressure. Blood samples were obtained via the jugular catheter.

After the collection of baseline data for 1 week, all of the pigs were treated with DOCA (200 mg/kg) via subcutaneous implants of DOCA-impregnated sili-

cone strips in the left flank. Surgery was conducted under halothane anesthesia and sterile conditions. MAP was followed for 3 weeks after DOCA implantation. Blood pressure measurement sessions before and after DOCA treatment were conducted twice a week with each session lasting for 15-20 minutes. Body weights were obtained at the end of each session. Blood samples were drawn weekly and were stored at -20°C until analysis. Throughout this phase of the study, both groups consumed the same amount of food; daily sodium intake was approximately 25 meq/day for all of the pigs.

During the last conscious blood pressure measurement session, each pig was tested with a bolus injection of tyramine hydrochloride (5.0 mg i.v.) to assess the effectiveness of sympathectomy. In addition, phenylephrine hydrochloride (100 µg i.v.) and sodium nitroprusside (0.5 mg i.v.) were administered to evaluate cardiovascular responsiveness.

At the end of the study, tissue samples from the left ventricle, gastrocnemius muscle, and the cortices of the left and right kidneys were obtained for the analysis of catecholamine content. Tissues were stored at -70°C until analyzed with high-performance liquid chromatography with electrochemical detection (Waters Chromatography Division, Milford, Mass.).

Sample Analysis

Plasma samples were used for the measurement of electrolytes and PRA. PRA was determined by radioimmunoassay for angiotensin I (Ang I) (New England Nuclear, Boston, Mass.). Plasma samples were analyzed for sodium and potassium by using flame photometry. Total hemoglobin was measured in whole blood with spectrophotometry according to the cyanmethemoglobin method (Sigma Chemical Co., St. Louis, Mo.). Tissue catecholamines were extracted and analyzed as previously described. Recovery averaged 50%. The detection limit for norepinephrine was 0.16 ng/g, and for epinephrine and dopamine it was 0.096 ng/g. Intra-assay and interassay coefficients of variation were 10.5% and 20.9%, respectively.

Statistical Analysis

Standard statistical techniques were used for the calculation of mean values and standard errors (SEM), which are given in the text and figures. Analysis of the group by period data was performed using a two-way analysis of variance (ANOVA) with repeated measures. When appropriate, a one-way ANOVA for repeated measures was used to evaluate changes among treatment periods within a group. Post hoc analysis was completed using Dunnett's test. For selective comparisons between control and 6-OHDA-treated animals, a one-way ANOVA was performed followed by Fisher's least significant difference. A difference was considered significant if \( p < 0.05 \).

Results

Overall, the animals tolerated the administration of 6-OHDA well. Acute, behavioral reactions to the
drug appeared within several minutes after injection and included lethargy, burrowing, shivering, excess salivation, disinterest in nursing, and occasionally vomiting. These behavioral responses decreased in intensity over time, and by the fifth week of 6-OHDA treatment, they were greatly diminished or absent.

Body weights were similar between the control and the 6-OHDA–treated swine from the time of birth until 4 weeks of age. From 5 weeks of age until the completion of the study, body weights were significantly lower in the 6-OHDA group. By the end of the study, the control pigs weighed 23 ± 2.5 kg and the 6-OHDA–treated pigs weighed 13 ± 0.7 kg (p < 0.05).

Conscious, resting blood pressures in the control and 6-OHDA–treated swine before and for 3 weeks after DOCA treatment are shown in Figure 1. After approximately 18 weeks of 6-OHDA treatment, but before DOCA administration, resting MAP in conscious animals was not significantly different between the 6-OHDA (116 ± 2 mm Hg) and control (125 ± 5 mm Hg) groups. Resting HR also was not significantly different between the two groups before DOCA treatment (6-OHDA, 111 ± 3; control, 97 ± 1 beats/min). One week after DOCA administration, MAP increased significantly by 20–22 mm Hg in both groups. During weeks 2 and 3 after DOCA administration, MAP continued to increase in the control group, reaching a value of 163 ± 6 mm Hg by week 3. In contrast, MAP in the 6-OHDA group did not rise further during this time period. MAP was significantly lower in the 6-OHDA group when compared with the control group at weeks 2 and 3 after DOCA administration.

Table 1 shows the MAP and HR responses to bolus injections of tyramine, phenylephrine, and nitroprusside in conscious swine. The pressor response to tyramine (5.0 mg i.v.) was significantly reduced by 95% in the 6-OHDA–treated group when compared with the control group. There was no change in HR in response to tyramine in either group. Phenylephrine (100 μg i.v.) elicited a similar increase in MAP in both the control and 6-OHDA–treated groups. The reflex bradycardia associated with the phenylephrine-induced increase in MAP was also similar between the two groups. Administration of nitroprusside (0.5 mg i.v.) caused a fall in MAP that was 94% greater in the 6-OHDA–treated group compared with the control group, despite a greater chronotropic response in the 6-OHDA–treated group.

There were no differences between the control and 6-OHDA–treated groups in hematocrit, plasma so-

Table 1. Hemodynamic Responses to Tyramine, Phenylephrine, and Nitroprusside in Six Conscious Control and Eight Sympathectomized Swine

<table>
<thead>
<tr>
<th>Test</th>
<th>ΔMAP (mm Hg)</th>
<th>ΔHR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>6-OHDA</td>
</tr>
<tr>
<td>Tyramine (5 mg)</td>
<td>12 ± 2</td>
<td>1 ± 2*</td>
</tr>
<tr>
<td>Phenylephrine (100 μg)</td>
<td>40 ± 3</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>Nitroprusside (0.5 mg)</td>
<td>-43 ± 7</td>
<td>-82 ± 6*</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; 6-OHDA, 6-hydroxydopamine.
*p < 0.05 vs. control.
Table 2. Tissue Catecholamine Content in Six Control Swine and Eight Swine Sympathectomized by Chronic Treatment With 6-Hydroxydopamine

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>284.4±149.9</td>
<td>5.4±0.6</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>9.0±3.7*</td>
<td>2.0±0.6*</td>
<td>0.6±0.2*</td>
</tr>
<tr>
<td>Right kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>239.3±16.1</td>
<td>5.9±0.8</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>16.3±9.4*</td>
<td>3.6±1.1</td>
<td>0.8±0.2*</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,465.7±193.8</td>
<td>53.3±5.1</td>
<td>26.7±5.5</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>5.1±1.8*</td>
<td>1.9±0.8*</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40.0±5.8</td>
<td>1.7±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>0.6±0.3*</td>
<td>1.0±0.4</td>
<td>†</td>
</tr>
</tbody>
</table>

All values are expressed in nanograms per gram of tissue. 6-OHDA, 6-hydroxydopamine.
*P<0.05 vs. control. †Below detection limits.

Discussion

These data indicate that an effective, peripheral sympathectomy of relatively long duration can be achieved in miniature swine when these animals are chronically treated with 6-OHDA beginning soon after birth. A variety of 6-OHDA dosages and treatment schedules have been used to chemically sympathectomize animals of different species. These have ranged from a single, large dose to repeated administration over a period of hours or days. The effectiveness of 6-OHDA depends on its initial uptake into the adrenergic nerve terminals, where, upon accumulation in a sufficiently high concentration, it exerts its toxic effects and causes destruction of the terminals. In adults, the cell bodies are not affected and regrowth of nerve fibers and recovery of adrenergic function begin within 4–5 days after cessation of treatment. Within 2 to 3 months, innervation may be restored to normal. Administration of 6-OHDA to neonates destroys the entire neuron, including cell bodies in the sympathetic ganglia, thereby resulting in what may be a more complete sympathectomy. Based on this information, we chose to begin treatment of our swine with 6-OHDA on the first day after birth and to administer graded doses of 25, 50, and 75 mg/kg over a period of several days. It has been recommended that weekly injections of 6-OHDA be given to maintain a sympathectomized condition. To insure that the highest degree of sympathectomy would be maintained in our animals, we chose to treat them with 6-OHDA for the entire duration of the study.

Chemical sympathectomy was confirmed in the 6-OHDA–treated swine by the lack of a pressor response to a bolus dose of intravenous tyramine and by depletion of tissue norepinephrine. In the control swine, tyramine produced a 12±2 mm Hg rise in MAP, presumably due to the release of norepinephrine from adrenergic nerve terminals. The response to tyramine was reduced by 95% in the 6-OHDA–treated group, indicating destruction of most, but possibly not all, of the nerve terminals. These results are similar to those obtained by other investigators.

A similar pressor response to the α-agonist phenylephrine in the conscious control and 6-OHDA–treated groups indicates that the sympathectomized swine were fully capable of responding to stimulation of α-receptors. Based on these results, we concluded that the lack of a pressor response to tyramine as described above was due to the absence of the...
neurotransmitter, not due to an inability of the \( \alpha \)-receptors to respond. Despite a 30% greater increase in HR, the 6-OHDA-treated swine experienced a nitroprusside-induced fall in MAP that was almost twice as great as that of the control group. The exaggerated fall in MAP in the 6-OHDA-treated group reflected severely impaired compensatory mechanisms, providing further indirect evidence that an effective peripheral sympathectomy was achieved.

In the DOCA-treated control pigs, renal and ventricular norepinephrine content was not significantly different from values we have observed in non-DOCA-treated miniature swine. Tissue norepinephrine content in the kidney, heart, and gastrocnemius muscle was reduced by greater than 93% with chronic 6-OHDA treatment in the present study. Epinephrine and dopamine were also found to be reduced by 96% and 98%, respectively, in the left ventricle. These data indicate disruption of the sympathetic innervation to these organs. It has been shown by several researchers that 6-OHDA does not affect all adrenergically innervated organs to the same extent.14-16 Also, the amount of nerve destruction and the time for regeneration varies from organ to organ and from species to species. Blood vessels seem to be particularly resistant to norepinephrine depletion by 6-OHDA, but it has been suggested that these differences could be overcome if a sufficient amount of 6-OHDA were to be used.17 Factors affecting the degree of sympathectomy attained with 6-OHDA include 1) a critical dose of 6-OHDA for destruction of nerve terminals, 2) the amount of blood flow to the organ, 3) the density of adrenergic innervation, and 4) the degree of maturation of nerve fibers at the time of treatment.6 Although we did not measure any vascular norepinephrine content in this study, the degree of norepinephrine depletion found in the tissues that were sampled (more than 93%) was highly indicative of an extensive sympathectomy due to chronic treatment of the animals with 6-OHDA. Based on the biochemical and functional tests described above, it can be concluded that administration of 6-OHDA according to the schedule used in this study is an effective means of achieving a generalized, peripheral sympathectomy in miniature swine. However, additional studies should be performed to determine if smaller and less frequent doses of 6-OHDA can be used to achieve an equally effective sympathetomy.

In the second part of our study, we were interested in evaluating the response of chemically sympathectomized swine to DOCA, which normally causes hypertension in these animals. Before DOCA treatment, MAP was slightly, although not significantly, lower in the 6-OHDA-treated group when compared with the control group. Similar slight decreases in resting blood pressure after 6-OHDA treatment have also been observed by others.23-25 The absence of a significant chronic decrease in MAP after sympathetic ablation could be explained by the activation of compensatory mechanisms, since redundancy exists among the blood pressure-regulating systems. Previous studies have shown that activity of the adrenal medulla may increase so that the effect of sympathectomy on resting blood pressure is minimized.6,9

In miniature swine, blood pressure becomes significantly elevated within 3-5 days after DOCA administration.19 DOCA hypertension in swine is characterized by elevated sympathetic nervous system activity and increased total peripheral resistance with no change in cardiac output.7-9 In the present study, the increase in MAP during the first week after DOCA treatment was similar in the 6-OHDA-treated and control groups. However, after week 1 the blood pressure curves diverged. The control group exhibited the steady rise in MAP over time in a manner typically seen with DOCA treatment, whereas in the 6-OHDA-treated group MAP plateaued at week 1 and remained at this level for the duration of the study. These results suggest that the absence of a peripheral sympathetic nervous system does not completely prevent a DOCA-induced rise in blood pressure. However, the full development of DOCA hypertension is not achieved in the absence of an intact peripheral sympathetic nervous system.

In other hypertensive animal models that exhibit increased sympathetic nerve activity, 6-OHDA also attenuated the severity of the hypertension. Chemical sympathectomy with 6-OHDA has been shown to attenuate the rise in blood pressure in the Okamoto strain of spontaneously hypertensive rats,20 in the New Zealand strain of genetically hypertensive rats,18 and in stroke-prone spontaneously hypertensive rats.21 Also, in Dahl salt-sensitive rats, 6-OHDA prevented the development of hypertension when the rats were fed a high salt diet.22 The possibility of a central effect contributing to the lower blood pressure in our 6-OHDA DOCA-treated swine and in these other hypertensive models should be considered, since it has been shown that 6-OHDA can cross the incomplete blood–brain barrier in neonates and cause depletion of norepinephrine in certain regions.15-16 In addition, intracerebroventricular administration of 6-OHDA has been shown to prevent the increase in MAP and plasma norepinephrine when rats are treated with DOCA and saline.23

The factors responsible for the initial increase in MAP in the 6-OHDA-treated group after DOCA implantation are unknown. The lack of complete prevention of the development of experimental hypertension when using 6-OHDA in some models has been attributed by others to an incomplete sympathetomy or to the rapid regeneration of vascular adrenergic nerves.14,17 Although we cannot exclude these possibilities because we did not directly assess the vascular nerves, they are unlikely explanations for these results considering the chronic treatment with 6-OHDA (4-5 months), depletion of norepinephrine in all tissues that were assayed, and the other strong indications of a peripheral sympathectomy that were discussed above. Because hypertension is multifacto-
rial in nature and mineralocorticoids are known to influence renal function, vascular reactivity, and vasopressin secretion,\textsuperscript{24} it seems likely that there is an interplay between both neural and nonneural components during the development of DOCA hypertension. By removing part of the neural component (i.e., sympathetic nervous system) we have altered, but not completely prevented, the development of mineralocorticoid hypertension. The fact that hemocrit and hemoglobin values did not change after DOCA administration in the 6-OHDA-treated swine suggests that the initial increase in MAP was not due to volume expansion.

In both the control and 6-OHDA-treated groups, MAP increased approximately 20 mm Hg the first week after DOCA. This increase in MAP, and hence renal perfusion pressure, is thought to be important for the animals to maintain normal sodium balance in the presence of mineralocorticoid.\textsuperscript{25} Sodium excretion in DOCA-treated swine is highly pressure dependent.\textsuperscript{7} When treated with DOCA, which normally causes hypertension and a rightward shift in the pressure-natriuresis curve, MAP in the 6-OHDA-treated group in this study stabilized at a value that was 28 mm Hg lower than that of the control group, yet the 6-OHDA-treated group did not appear to be retaining sodium (i.e., no evidence of peripheral edema or ascites). These results provide indirect evidence that the sympathetic nervous system plays a role in determining the position of the pressure-natriuresis curve in DOCA-treated miniature swine.

In summary, we have shown that chronic treatment with 6-OHDA from birth produced an effective, general, peripheral sympathectomy in miniature swine. This sympathetomized condition was characterized by a greatly attenuated pressor response to tyramine and by depletion of tissue catecholamines. In addition, chemical sympathectomy significantly attenuated the normal rise in blood pressure that is seen in swine that are treated with DOCA. These results support the hypothesis that an intact sympathetic nervous system is necessary for the full expression of hypertension in DOCA-treated miniature swine.

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References


\textbf{KEY WORDS} • 6-hydroxydopamine • sympathetic nervous system • catecholamines • animals, newborn

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