Effects of 6-Hydroxydopamine on the Canine Sinus Node

By Victor Elharror, Jacques de Champlain, and Réginald A. Nadeau

ABSTRACT
The acute effects of 6-hydroxydopamine (6-OH-DA) injected directly into the sinus node artery were studied in 57 dogs. The positive chronotropic effect of 6-OH-DA was intermediate between that of norepinephrine and that of tyramine, when these substances were compared on the basis of their ED50. The increase in heart rate produced by 6-OH-DA was inhibited by prior intranodal injection of propranolol and desmethylimipramine. At high doses, injection of 6-OH-DA was often associated with arrhythmias such as periodic slowing of the sinus rate, atrial premature beats, atrial fibrillation, and ventricular extrasystoles. In the 72 hours following injection of 1.5 mg 6-OH-DA, the norepinephrine content of the sinus node region decreased progressively. In agreement with the norepinephrine depletion, response to intranodally injected tyramine was significantly diminished. The number of fluorescent adrenergic nerve fibers was also reduced. Impaired norepinephrine uptake and enhanced release were also demonstrated by using tritiated norepinephrine. After 6-OH-DA treatment, response to right stellate stimulation was diminished, and the response to vagal stimulation was not altered. It was concluded that the local perfusion of the sinus node with 6-OH-DA produces a marked norepinephrine depletion of the perfused region compatible with a localized degeneration of the sympathetic nerve terminals.

KEY WORDS: adrenergic nerve terminals, cardiac arrhythmias, right stellate ganglion stimulation, tyramine, histofluorescence, uptake and release of norepinephrine

Studies on the autonomic innervation of the heart indicate that this organ is richly innervated but that innervation of the sinoatrial and atrioventricular nodes is denser than that of other cardiac regions (1, 2). The role of the adrenergic system in the control of the heart rate and contractility has been studied by various procedures such as surgical denervation, immunosympathectomy, or use of substances interfering with norepinephrine synthesis, storage mechanisms, or the action on receptors. Each of these techniques has some disadvantages in producing incomplete sympathetic denervation, requiring high technical skill, or affecting functions unrelated to the sympathetic system. Recent studies (3-5) have shown that 6-OH-DA produces a specific degeneration of adrenergic nerve terminals, without affecting surrounding Schwann cells, smooth muscle cells, and cholinergic nerve terminals. This compound constitutes a new means for producing sympathectomy and thus provides a more specific approach to evaluate the role of the sympathetic nervous system. The possibility of producing a local adrenergic denervation of the sinus node was studied by injecting 6-OH-DA into the sinus node artery. The effects of 6-OH-DA on heart rate, metabolism of norepinephrine, and disappearance of fluorescence of adrenergic nerves were
investigated at various times after injection.

Methods

Fifty-seven mongrel dogs weighing 10 to 15 kg were anesthetized with sodium pentobarbital (35 mg/kg iv). After tracheal intubation, the animal was maintained under assisted respiration with a Harvard pump, the chest opened at the level of the 4th intercostal space, and the pericardium incised. The sinus node artery was dissected at its origin and cannulated with siliconized polyethylene tubing, as described by James and Nadeau (6). The responsiveness of each preparation was tested by injecting acetylcholine into the sinus node artery, and only preparations responding by sinus arrest to a dose of 1 μg or less of acetylcholine were retained for experimentation.

The right stellate ganglion was exposed and prepared for stimulation with bipolar silver electrodes. Square wave stimuli of 2 msec duration and of supramaximal voltage were delivered from a Grass S4 stimulator at frequencies of 1, 3, 10, and 30 Hz. In other experiments the right and left vagus nerves were exposed and severed in the neck. The distal end of the right vagus nerve was stimulated in the same way as the right stellate ganglion.

A right atrial electrogram obtained from a small unipolar silver pin electrode fixed to the right atrial appendage was recorded simultaneously with a peripheral electrocardiogram (lead AV R). Electrical and blood pressure tracings were recorded on a direct writer Brush polygraph. Heart rate was computed by a digital tachometer triggered by the right atrial electrogram and recorded on a direct writer Brush polygraph.

In dogs kept 24 and 72 hours after intranodal injection of 6-OH-DA, the cannula was removed and the sinus node artery ligated. Sinus node activity was unaffected by ligation of this artery, as previously reported (6). The pericardium was sutured and the chest closed.

The following drugs were used: Z-arterenol-D-malate monohydrate (Mann Research Laboratories), tyramine-HCl (Nutritional Biochemicals Corp.), acetylcholine chloride (British Drug House, Ltd.), desmethylimipramine (Parke-Davis, Geigy Canada, Ltd.), propranolol (Ayerst Laboratories) and 6-hydroxydopamine-HBr (Regis Chemical Co.). Various concentrations of these drugs were prepared in fresh Ringer’s solution. For 6-OH-DA, ascorbic acid at a concentration of 1 mg/ml was added to the final solution. Injections of 1 ml were made into the sinus node artery from a hand-driven syringe in about 30 seconds.

When serial injections of increasing concentrations were made, the heart rate was permitted to return to the preinjection level for at least 5 minutes before the following injection. Between injections of various drug concentrations, the cannula was washed with 1 ml of Ringer’s solution. Drug concentrations are expressed as the salt, with the exception of those in Figure 1, where they are expressed as the base to facilitate comparison between 6-OH-DA, tyramine, and norepinephrine.

Endogenous and tritium-labeled norepinephrine contents were determined on samples of heart obtained from the following regions: the sinus node, presinus node, postsinus node, superior vena cava, tips of the right and left atrial appendages, and the left ventricular apex. The presinus node region consisted of a fragment of the anterior right atrial wall containing the sinus node artery from the point of cannulation halfway to the atrio caval junction. The sinus node region consisted of a part of the right atrium extending cephalad to the presinus region, including the atrio caval junction at the upper margin of the right atrial appendage. The superior vena cava fragment was obtained just above the atrio caval junction. No attempt was made to evaluate histologically the proportion of conduction tissue contained in any of these fragments.

Tritiated norepinephrine hydrochloride (8 c/mmole) (New England Nuclear, Boston, Mass.) was purified by alumina column chromatography before injection into the sinus node artery. At the time of sampling, tissue fragments were quickly dissected, chilled on crushed ice, homogenized in 10 ml of 0.4N perchloric acid, and analyzed for radioactive and endogenous norepinephrine. Alumina and tissues were prepared according to the method of Anton and Sayre (7). After titration with NaOH to pH 8.6, the perchloric acid extract of tissue was poured on a column (0.6 cm x 0.4 cm) containing 400 mg of alumina. The column was washed with 5 ml of 0.2N Na acetate and 10 ml of glass-distilled water, the effluent and wash were discarded and norepinephrine was eluted with 6 ml of 0.2N acetic acid. The radioactivity in 0.2 ml aliquots of the perchloric acid extract and of the alumina eluate was measured in a liquid scintillation spectrometer after addition of 4 ml of ethan - methanol mixture (3:1) and 10 ml of toluene-phosphor. In this system, the counting efficiency for tritium was 10%. The values for 3H-norepinephrine were corrected for a recovery of 80%. An estimate of the total 3H-metabolites was obtained by calculating the difference between the radioactivity of the perchloric acid homogenate and the radioactivity of the acetic acid eluate from the alumina. Aliquots of 0.5 ml of the
eluate were used for the fluorometric determination of norepinephrine. The endogenous norepinephrine was converted to trihydroxyindoles through oxidation by potassium ferricyanide at pH 6.5, according to the method of von Euler and Lishajko (8). The norepinephrine values were corrected for a recovery of 90%.

Samples of the sinus and presinus node regions were prepared for fluorescence microscopy, according to the technique of Falck et al. (9). Tissues were quickly frozen in propane liquified by circulation through a copper coil immersed in a bath containing a mixture of dry ice and acetone. The frozen tissues were transferred to a special freeze-dryer apparatus (10) and dessicated for 3 days at a temperature of —30°C. The dehydrated tissues were exposed to paraformaldehyde vapor at optimum humidity for 1 hour at 80°C (11). The tissues were then vacuum-embedded in paraffin and 8-μm sections were mounted in Eukitt (Otto C. Watzka Co. Ltd., Montreal). The fluorescence microscopy was made with a Leitz Orthomat automatic microscope coupled with an Osram HBO 200 lamp using the appropriate set of filters (activation 400 μm, secondary filter 510 μm).

Student's t-test was used for statistical analysis.

Results

Acute Chronotropic and Arrhythmic Effects

The intranodal injection of 6-OH-DA caused an immediate increase in heart rate. A maximum response was obtained after injecting 1.5 mg. The dose-response curve for 6-OH-DA is shown in Figure 1 and is compared to those obtained with norepinephrine and tyramine. The slope of the three curves and the maximal response are similar for all three substances. The ED50 for norepinephrine, 6-OH-DA, and tyramine were respectively 2 × 10⁻⁷, 6 × 10⁻⁶, and 3 × 10⁻⁵ g/ml. The peak chronotropic effect of 6-OH-DA was dose-related (Table 1) and was significantly (P < 0.01) longer lasting than that obtained from equivalent doses of norepinephrine and tyramine.

The acute chronotropic response to 6-OH-DA could be prevented by blockade of β-receptors and by procedures interfering with the neuronal uptake mechanisms. Injection of propranolol (100 μg) or of desmethylimipramine (2 × 5 μg) prior to the injection of 6-OH-DA completely prevented the expected increase in heart rate.

During the initial phase of sinus tachycardia, four types of arrhythmic phenomena were observed: periodic slowing of the sinus node, atrial premature beats, atrial fibrillation, and ventricular extrasystoles. Figure 2 shows an example of periodic slowing of sinus rhythm 2 minutes after injecting 1.5 mg of 6-OH-DA into the sinus node artery and interrupting the sinus tachycardia. This arrhythmia was never observed in vagotomized dogs but was observed in 50% of the dogs with intact vagi. The phenomenon of periodic slowing of the sinus rate appeared to be associated with a rise in blood pressure after injecting 1.5 mg of 6-OH-DA into the sinus node artery. In 12 dogs.

Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>n</th>
<th>Min</th>
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<tr>
<td>15 μg</td>
<td>4</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>150 μg</td>
<td>5</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>15000 μg</td>
<td>18</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>15000 μg</td>
<td>4</td>
<td>150 ± 9</td>
</tr>
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receiving an injection of 1.5 mg of 6-OH-DA into the sinus node artery, the mean blood pressure increased from 115 ±15 (SE) to 160 ± 25 mm of Hg. In ten dogs observed after an injection of 150 μg of 6-OH-DA the blood pressure did not rise significantly and periodic slowing of the sinus rate did not occur.

Atrial premature beats were noted in 35 of the 43 dogs studied after injections of 150 μg and 1.5 mg of 6-OH-DA (Table 2). A greater number of atrial premature beats occurred with the higher dose. Of the 35 dogs with atrial premature beats, 22 had episodes of atrial fibrillation of short duration (60 ± 20 sec). Atrial fibrillation was invariably initiated by atrial premature beats early in the cardiac cycle. In dogs that did not have atrial premature beats, atrial fibrillation was not observed. The section of the vagus did not change the incidence of atrial fibrillation.

Ventricular extrasystoles were observed in three dogs after injection of 6-OH-DA. One animal had received 1.5 mg of 6-OH-DA into the sinus node artery; the two others had received 15 mg.

**EFFECTS ON NOREPINEPHRINE METABOLISM**

Four groups of dogs were injected with 1.5 mg of 6-OH-DA into the sinus node artery and killed 2, 5, 24, or 72 hours later. A fifth group, killed 2 hours after injection of 1 ml of Ringer's solution, served as control. The norepinephrine content was measured in the seven cardiac fragments sampled as described in Methods. The norepinephrine content was decreased more specifically in the sinus and pre-sinus node regions (Table 3). A progressive depletion occurred in the sinus node.
Time course of norepinephrine depletion of the sinus node region after injection of 1.5 mg of 6-OH-DA into the sinus node artery. Each point is the mean value ± SE of 3 to 6 dogs. P < 0.05 at 2 hours and <0.01 for the remaining points.

region during the first 72 hours following the injection (Fig. 3). The depletion proceeded more rapidly in the first 5 hours and continued at a slower rate during the following hours. After 3 days, the norepinephrine content of the sinus node and presinus node regions were lowered to 15% and 4% of the respective control values. Regions of the heart adjacent to the sinus node region, such as the postsinus node and superior vena cava, also showed a decreased norepinephrine content, but other remote regions were affected to a lesser degree (Table 3). Although the endogenous norepinephrine concentration decrease is only 50% 2 hours after 6-OH-DA treatment, the number of fluorescent adrenergic fibers appears to be markedly reduced in the sinus node (Fig. 4).

The chronotropic response to injections of tyramine into the sinus node artery was studied in a group of five dogs treated 2 hours previously with intranodal injection of 6-OH-DA (1.5 mg) and was markedly reduced both in amplitude (P < 0.01) (Fig. 5) and duration at all the doses tested. Control dose-response curves to tyramine (1, 10, 100, and 1000 μg) were obtained in the same dogs before administration of 6-OH-DA. Repeated injection of 10 μg of tyramine, 1 hour after determination of the control curve, gave the same response as observed with the initial 10

<table>
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<th>TABLE 3</th>
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<tr>
<td><strong>Endogenous Norepinephrine (μg/g Tissue)</strong></td>
</tr>
<tr>
<td>Region</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Sinus node</td>
</tr>
<tr>
<td>Presinus node</td>
</tr>
<tr>
<td>Postsinus node</td>
</tr>
<tr>
<td>Superior vena cava</td>
</tr>
<tr>
<td>Right atria</td>
</tr>
<tr>
<td>Left atria</td>
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<tr>
<td>Apex</td>
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*1.5 mg injected into sinus node artery; Each value is the mean ± se of six dogs. 1P < 0.05. 1P < 0.01.

Circulation Research, Vol. XXVIII, February 1971
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Sympathetic innervation of the sinus node region. A shows normal innervation of a control dog. B shows decrease of fluorescent adrenergic nerve terminals 2 hours after injection of 1.5 mg of 6-OH-DA into the sinus node artery.

FIGURE 4

Dose-response curve to tyramine injected into the sinus node artery before and after 6-OH-DA once heart rate had returned to control level. Each point is the mean ± SE of 5 dogs. P < 0.01 for all the points.

FIGURE 5

Mu
g, thus suggesting that no tachyphylactic phenomenon results from determination of the control curve to tyramine and that 6-OH-DA is responsible for the reduced effect of tyramine.

The decreased norepinephrine content of heart tissue after 6-OH-DA is in part explained by an increased release of norepinephrine. This was shown by labeling the sinus node with tritiated norepinephrine 15 minutes before injecting 6-OH-DA in the experimental group or Ringer's solution in the control group. Only 15% of the control value of tritiated norepinephrine was found in the sinus node region of the dogs injected with 6-OH-DA. As estimated by percent of the total counts not attributable to 3H-norepinephrine, the proportion of metabolites appeared to be increased in dogs given 6-OH-DA (Table 4). Decreased specific activity of norepinephrine was observed in the sinus and presinus node regions after treatment with 6-OH-DA. During the same period, endogenous norepinephrine was decreased by 33%, whereas radioac-
Table 4

Effect of 6-OH-DA on NE Release Studied with Tritiated Norepinephrine

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>After 6-OH-DA</th>
</tr>
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<tbody>
<tr>
<td>Sinus node</td>
<td>91,600 ± 31,800</td>
<td>15,310 ± 8,000*</td>
</tr>
<tr>
<td>Pre sinus node</td>
<td>105,760 ± 35,600</td>
<td>33,930 ± 18,800*</td>
</tr>
<tr>
<td>NE Content (μg/g Tissue)</td>
<td>4.03 ± 0.30</td>
<td>2.66 ± 0.48</td>
</tr>
<tr>
<td>Specific Activity (counts/min/μg NE)</td>
<td>2.24 ± 0.24</td>
<td>2.12 ± 0.28*</td>
</tr>
<tr>
<td>Sinus node</td>
<td>2.2 ± 0.85 × 10^-6*</td>
<td>0.62 ± 0.35 × 10^-6*</td>
</tr>
<tr>
<td>Pre sinus node</td>
<td>2.59 ± 0.85 × 10^-6*</td>
<td>1.32 ± 0.62 × 10^-6*</td>
</tr>
<tr>
<td>% Total Count Recovered as NE</td>
<td>91.2 ± 7.4</td>
<td>52.3 ± 9.3*</td>
</tr>
<tr>
<td>Pre sinus node</td>
<td>92.2 ± 3.0</td>
<td>58.7 ± 9.9*</td>
</tr>
</tbody>
</table>

Tritiated norepinephrine (2 μc) was injected 15 minutes before injection of 1.5 mg of 6-OH-DA into the sinus node artery. The animals were killed 2 hours later.

Values are means ± SE of six dogs.

*P < 0.01. **P < 0.05.

Impaired norepinephrine uptake was demonstrated by injecting tritiated norepinephrine 5 hours after injection of 6-OH-DA or Ringer's solution into the sinus node artery. The norepinephrine uptake was decreased by 55% in the sinus node region and by 80% in the pre sinus node region of the dogs previously treated with 6-OH-DA (Table 5).

**CHRONOTROPIC RESPONSE TO SYMPATHETIC AND PARASYMPATHETIC NERVE STIMULATION**

The chronotropic response to stimulation of the right stellate ganglion was studied in vagotomized preparations following intranodal injections of various doses of 6-OH-DA (15, 150, 1500, and 15,000 μg). The response is impaired by 85% (Table 4).

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Figure 6

Chronotropic response to various frequencies of stimulation of the right stellate ganglion after various doses of 6-OH-DA and after heart rate had returned to its control level. Results are expressed as mean value of the increase of heart rate ± SE. Each group contained 5 animals. *P < 0.01 for 1500 μg and 15,000 μg.
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The sympathetic response was markedly reduced at all stimulation frequencies after injection of 1.5 and 15 mg, but not significantly reduced after a dose of 150 μg (Fig. 6). During the peak of sinus tachycardia, the response to stellate stimulation appeared to be abolished. However, as the heart rate returned to its control level, the response to stellate stimulation recovered partially then decreased with time (Fig. 7).

In contrast to the reduced response to sympathetic stimulation, the response to stimulation of the distal end of the right vagus was unchanged following injection of 1.5 mg and 15 mg of 6-OH-DA in five and two dogs, respectively.

Discussion

The results of the present study show that 6-OH-DA injected directly into the sinus node artery produces a long-lasting chronotropic effect along with marked reduction in the norepinephrine content and in the number of fluorescent adrenergic nerve terminals of the sinus node region. The acute sympathomimetic response and the norepinephrine depletion can be explained by a massive norepinephrine release from the adrenergic nerve endings as well as by impaired norepinephrine uptake.

Sinus tachycardia has been reported by other investigators in different species following intravenous injection of 6-OH-DA (5, 12, 13). It was proposed that this chronotropic effect results mainly from the liberation of catecholamines from the adrenal medulla (5). The technique used in the present study does not rule out a participation of the adrenal medulla but suggests that the release of norepinephrine arises mainly from local adrenergic nerve terminals. The sympathomimetic effect of 6-OH-DA can be prevented by first treating with desmethylimipramine, which interferes with neuronal uptake mechanisms; this suggests that 6-OH-DA has to be taken up by nerve terminals to release endogenous norepinephrine.

A decrease in the specific activity of norepinephrine was observed after injection of 6-OH-DA. This decrease is consistent with the idea that 6-OH-DA is taken up by nerve terminals to release endogenous norepinephrine.
Comparison of chronotropic responses to right stellate stimulation at a frequency of 30 cps and to intranodal injection of norepinephrine (10 μg) after increasing doses of 6-OH-DA. Response to stimulation is diminished significantly (P < 0.05) more than response to injected norepinephrine at 15,000 μg.

The norepinephrine-inactivating enzymes catecholamine O-methyl transferase and monoamine oxidase show only a slight decrease in activity after either chemical (13) or surgical denervation (17). Due to an enhanced release from its binding sites by 6-OH-DA, norepinephrine is more readily available for inactivating enzymes, thus accounting for the higher proportion of norepinephrine metabolites. The chronotropic response to right stellate stimulation was markedly reduced after 6-OH-DA treatment. Decreased responsiveness to sympathetic stimulation was also reported by Thoenen et al. (18) and Laverty et al. (5) in dogs and kittens 24 hours after intravenous injection of 6-OH-DA. Laverty and co-workers further reported that the response to sympathetic stimulation was abolished immediately after injection of 6-OH-DA. We also observed that soon after injection of 6-OH-DA, at a time when the heart rate was at its maximum, no further increase in heart rate could be obtained by stimulating the right stellate ganglia. The results of Laverty et al. could be explained on those terms since the dose they used was sufficient to cause a
tachycardia of maximal amplitude. Quantitative analysis of the reduced responsiveness to sympathetic stimulation was difficult to determine, since injected norepinephrine was also shown to be less active after 6-OH-DA although the responsiveness to injected norepinephrine was diminished to a lesser degree \((P < 0.05\) only at 15,000 \(\mu g\)) than the responsiveness to stellate stimulation. Moreover, analysis is complicated by the fact that the concentration of neurotransmitter at the surface of the pacemaker cells in the sinus node after sympathetic stimulation could be greater than that obtained after injection of 10 \(\mu g\) of norepinephrine (19). The reduced responsiveness to sympathetic stimulation in the present study is in part due to reduced effect of norepinephrine and also to a decrease of the amount of norepinephrine released by nerve terminals, as suggested by other investigators (18). Supersensitivity to norepinephrine as early as 2 hours after 6-OH-DA treatment has been reported (20, 21) on isolated rat atria and cat, but no reports are available on the sensitivity at an earlier time. This early subsensitivity could possibly be attributed to a failure of the sinus node cells to respond to exogenous norepinephrine after a prolonged hyperactivity or to a partial blockade of \(\beta\)-receptors by 6-OH-DA.

During the peak chronotropic effect from 6-OH-DA, a periodic slowing of sinus rhythm was observed in some dogs. The transitory character of this phenomenon rendered its investigation difficult. Langer and Trendelenburg (22) observed that after surgical denervation, the nictitating membrane shows burst activity in the early stage of the degeneration contraction. On the other hand, a similar cyclic variation in the sinus rate has been reported by Hawkins et al. (23) on dog heart-lung preparations and isolated guinea pig atria after administration of veratramine in the presence of norepinephrine. James and Nadeau (24) also reported a similar arrhythmia after injecting veratridine into the sinus node artery. Langer and Trendelenburg have suggested that after surgical denervation of the nictitating membrane, the release of norepinephrine from degenerating nerve terminals might occur in bursts or paroxysms. A similar phenomenon could possibly occur at the level of the sinus node when norepinephrine or of 6-OH-DA is released from adrenergic nerve terminals by 6-OH-DA. However, since this arrhythmia was not observed in vagotomized dogs and was always associated with a rise in arterial blood pressure, reflex activation of the vagi seems to be implicated. From the work of Hawkins and co-workers it is clear that a vagal reflex does not play a role in periodic slowing of the sinus node induced by veratrum alkaloids. These workers suggested that the mechanism involved in this type of arrhythmia is due to an antagonism between substances of opposite effect (i.e., veratramine-norepinephrine) on the sinus node cells. Although the action of acetylcholine in the presence of large amounts of norepinephrine or of 6-OH-DA is still unsettled, such an antagonism between acetylcholine and norepinephrine on the sinus node cells might explain this arrhythmia.

The high incidence of atrial fibrillation observed after administration of 6-OH-DA seemed to be related to the occurrence of atrial premature beats during the vulnerable period of the atria (25, 26). Similar phenomena were observed after injecting a high dose of norepinephrine into the sinus node artery (unpublished observations). Both appearance of atrial premature beats and the increased atrial vulnerability may be secondary to the local release of large amounts of norepinephrine by 6-OH-DA. Hoffman and Singer (26) and Hashimoto and co-workers (27) reported that catecholamines increase the vulnerability of the atria to fibrillation by changing automaticity, conduction, and refractoriness.

Acknowledgment

We wish to thank Miss M. R. Corbeil and Miss S. Imbeault for their valuable technical assistance.

References


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Circ Res. 1971;28:188-198
doi: 10.1161/01.RES.28.2.188

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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