Abnormal Cardiac Norepinephrine Storage in Isoproterenol-Treated Rats

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ABSTRACT
Repeated administration of large doses of isoproterenol to rats produced myocardial enlargement and a fall in blood pressure. A defect in storage of norepinephrine (NE) was manifested by a decrease in endogenous NE, unimpaired uptake of $^{3}H$-NE, and an accelerated rate of loss of accumulated $^{3}H$-NE. This accelerated loss of $^{3}H$-NE was characterized by a more rapid disappearance of $^{3}H$-NE from the granular or microsomal than from the supernatant NE fraction. The increased rate of loss of $^{3}H$-NE could be reversed by treatment with a ganglionic blocking agent, chlorisondamine, by salt restriction and mercurial diuresis, and by withholding isoproterenol treatment for 7 days. The NE storage defect resembled that previously described in the hearts of rats made hypertensive by administration of desoxycorticosterone and NaCl. It is possible that the NE storage defect in the hearts of isoproterenol-treated rats is the result of a combination of changes in sympathetic neural activity and altered neuronal electrolytes.

ADDITIONAL KEY WORDS
systolic blood pressure
myocardial enlargement

Myocardial hypertrophy can be produced after repeated administration of isoproterenol (1, 2). Recent studies have demonstrated that rats made hypertensive by unilateral nephrectomy and the administration of desoxycorticosterone trimethylacetate (DOCA) and NaCl develop myocardial hypertrophy and an alteration in the neuronal storage of norepinephrine (NE) (3, 4). This defect in catecholamine storage appears to be a reflection of the altered cardiac electrolytes, an increase in sympathetic nervous activity, or both (5). Electrolyte changes have been observed in the hearts of the isoproterenol-treated rats (2, 6), which resemble those found in the hearts of rats made hypertensive with DOCA and NaCl (7). Since treatment with isoproterenol (8) and DOCA and NaCl hypertension (9) are both accompanied by a fall in endogenous NE, we examined cardiac NE storage mechanisms of isoproterenol-treated rats to see if there are other similarities in NE storage in these two conditions. In contrast to the hypertensive animals, rats treated with isoproterenol have a reduced blood pressure. Consequently, isoproterenol-treated rats could be used to study amine storage mechanisms of enlarged hearts in rats whose blood pressure is low.

Our results demonstrate that there is a defect in the ability of the hearts of isoproterenol-treated animals to store NE. Moreover, this defect in NE storage is reversible and closely resembles that present in the animal made hypertensive with DOCA and NaCl. The alterations in both conditions may be the result of a combination of increased sympathetic nervous activity and intraneuronal ionic imbalance which alters the granular NE storage sites. A preliminary report of these findings has been presented elsewhere (10).

Methods
Male Sprague-Dawley rats weighing 100 to 150 g were given either 7.5 mg/kg isoproterenol hydrochloride or 0.2 ml 0.9% saline subcutaneously at 8 AM and 4 PM daily for 4 days and were used at 8 AM on the fifth day. Blood pressure was determined daily for 4 days in one group of control and one group of isoproterenol-treated
rats before the first isoproterenol dose of that day, as described previously (8). Although the hearts were not examined microscopically, no areas of gross infarction or color change were obvious on examination and no pathological evidence of congestive heart failure was found at the time of killing the isoproterenol-treated rats.

For studies of the turnover of NE, rats were given 10 or 25 μC of d-l-3H-norepinephrine (3H-NE), 9.4 c/mM, iv (New England Nuclear, purified before use by adsorption on alumina) at zero time, and were killed by cervical dislocation at the indicated time intervals. The hearts were rapidly removed, chilled on cracked ice, weighed, and homogenized in 10 ml 0.4N HCl. Endogeneous NE and 3H-NE were analyzed, and the turnover rates were estimated by previously described methods (4, 11-13). To determine the subcellular distribution of NE and 3H-NE, hearts were homogenized in 10 ml 0.25M sucrose containing 0.001M magnesium chloride and 0.005M phosphate buffer (pH 7.4) and fractionated as described previously (4, 14). Protein was determined by the method of Lowry et al. (15). The percent of cardiac output delivered to the heart was measured using K42 as described by Sapirstein (16). Chlorisondamine hydrochloride, a ganglionic blocking agent, was kindly supplied by Ciba Pharmaceutical Company. The sodium-free diet and control diet were obtained from Nutritional Biochemical Corporation. Mercuric cyanhydrin was purchased from Lakeside Laboratories, Inc.

Results

Effect of Isoproterenol Administration on Blood Pressure.—Although the hypotensive effect of a single dose (80 mg/kg subcutaneously) of isoproterenol terminated within 3 hours (2), repeated administration of isoproterenol (7.5 mg/kg) resulted in a decrease in systolic pressure which persisted for at least 16 hours (Fig. 1).

In rats treated with isoproterenol hydrochloride, 7.5 mg/kg subcutaneously, twice daily for 4 days, the mean wet weight of 23 hearts was 840 ± 19 mg. An equal number of hearts from rats that received 0.2 ml of 0.9% saline instead of isoproterenol weighed 550 ± 11 mg (P < .001). The total cardiac protein of eight hearts from rats that received isoproterenol as above was 90 ± 4 mg; eight saline-injected control rat hearts contained 80 ± 3 mg protein (P > .05). Thus a major portion of the increased weight was probably due to an increased water content. Sixteen hours after the last dose of isoproterenol or saline, estimation of the coronary blood flow relative to cardiac output using K42 did not reveal any difference between isoproterenol-treated and control animals.

Effect of Isoproterenol Administration on the Rate of 3H-NE Disappearance.—Control rats and rats treated with isoproterenol for 4 days prior to use were given 25 μC of 3H-NE iv at zero time and killed 5 minutes, 12, 18, 24, and 36 hours later. Although the value for cardiac 3H-NE specific activity after 5 minutes is higher (P < .05) in the isoproterenol-treated animals, the values are significantly less at the subsequent four time periods (Fig. 2, top). The time required for loss of half of the initial concentration in the isoproterenol-treated animals (5.5 hours) is significantly less than that of control animals (15 hours). Computation of the slopes, excluding the 5-minute values, indicated that in this later period the slopes are not significantly different (Fig. 2, top). To evaluate alteration in the disappearance curve at the earlier time periods, the experiment was repeated, using
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**FIGURE 2**

$^3$H-NE disappearance from the hearts of control and isoproterenol-treated rats. Sixteen hours after the last injection of isoproterenol or saline (see Methods) rats received 25 μc (top) or 10 μc (bottom) of $^3$H-NE (9.2 c/mM), and were killed at the indicated time intervals, and the specific activity of cardiac $^3$H-NE was determined. The mean ± SEM of four to seven rats at each time interval are given.

**FIGURE 3**

Time-dependent changes in subcellular distribution of cardiac $^3$H-NE in control and isoproterenol-treated rats. Sixteen hours after the last injection of isoproterenol or saline (see Methods) all rats received 10 μc $^3$H-NE, 9.2 c/mM (see bottom of Fig. 2) and were killed at the indicated time intervals, and the ratio of the specific activity (SA) of $^3$H-NE in the supernatant fluid (S) to that in the granular (G) fraction was determined. The means ± SEM of five or six observations are given. **P < 0.01.

Only 10 μc of $^3$H-NE (Fig. 2, bottom). The curves were found to intersect at about 1 hour, with the 5-minute value again significantly increased, and the 4-hour value decreased from the corresponding control values. Thus the rate of disappearance of $^3$H-NE from isoproterenol-treated rat hearts is only increased in the first few hours after $^3$H-NE administration. There was no change in the significantly lower concentration of endogenous NE in the isoproterenol-treated rats during the time period involved in either experiment.

**Time Course of Decline in Specific Activity of Cardiac Subcellular Fractions.**—Since the differences in the rate of disappearance of $^3$H-NE from control and isoproterenol-treated rat hearts were most marked in the 4 hours after $^3$H-NE administration, the specific activities of the NE present in the granular, or high-speed sedimentable fraction, and the high-speed supernatant fraction were determined. The ratio of specific activity of $^3$H-NE in the soluble fraction to the specific activity in the granular fraction was not different 5 minutes or 1 hour after $^3$H-NE administration, but by 4 hours the value of the ratio in isoproterenol-treated rat hearts was significantly higher ($P < 0.01$) than the corresponding control (Fig. 3). The difference appeared to be a consequence of a more rapid loss of $^3$H-NE from the granular fraction than from the soluble fraction (Fig. 4, Table 1).

**Restoration of Normal NE Storage Mechanisms in Isoproterenol-Treated Rat Hearts.—**Since the NE storage defect in the isoproterenol-treated rats appeared to resemble that previously described for the rat made hypertensive with DOCA and NaCl (3), and since chlorisondamine-induced ganglionic blockade can abolish the defect in hypertensive rats (6), the effect of this agent was examined in isoproterenol-treated and control rats. Eight hours after administering 10.
Time-dependent changes in subcellular distribution of cardiac \( ^{3} \text{H}-\text{NE} \) in control and isoproterenol-treated rats. Animals were treated as described in Figure 3. The difference in the mean value of the specific activity (SA) of \( ^{3} \text{H}-\text{NE} \) in each fraction (see Methods) at 5 minutes and 4 hours after intravenous administration is expressed as a percent of that observed at 5 minutes (see Table 1).

Moreover, the difference \((P < .01)\) between the specific activities of control \((107 \pm 19)\) and isoproterenol-treated \((104 \pm 11)\) rats that did not receive chlorisondamine was abolished in animals that received this ganglionic blocking agent \((\text{control} 229 \pm 10; \text{isoproterenol-treated} 208 \pm 40)\).

When the retention of \( ^{3} \text{H}-\text{NE} \) in isoproterenol-treated rat hearts was examined 7 days after the end of the isoproterenol-treated period, the storage defect was not evident (Table 2). The initial uptake \((\text{at 5 minutes})\) of \( ^{3} \text{H}-\text{NE} \) was not significantly different at this time. Thus, the storage defect is reversible within 7 days.

Restriction of sodium intake can reverse the NE storage defect in DOCA and NaCl hypertensive rats. 

\( ^{3} \text{H}-\text{NE} \), iv, there was less \( ^{3} \text{H}-\text{NE} \) remaining in the hearts of isoproterenol-treated rats than in the hearts of control rats. Administration of chlorisondamine hydrochloride, 10 mg/kg subcutaneously, 5 minutes after labeling the amine stores with \( ^{3} \text{H}-\text{NE} \) resulted in an increase in the specific activity of \( ^{3} \text{H}-\text{NE} \) remaining 8 hours later in both control and isoproterenol-treated rats (Table 2).

Moreover, the difference \((P < .01)\) between the specific activities of control \((167 \pm 19)\) and isoproterenol-treated \((104 \pm 11)\) rats that did not receive chlorisondamine was abolished in animals that received this ganglionic blocking agent \((\text{control} 229 \pm 10; \text{isoproterenol-treated} 208 \pm 40)\).

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Restriction of sodium intake can reverse the NE storage defect in DOCA and NaCl hypertensive rats. Therefore, the retention of \( ^{3} \text{H}-\text{NE} \) was determined in isoproterenol-treated rats given 56 sucrose in distilled water, a sodium-free diet for 24 hours before, and a mercurial diuretic (0.1 ml of mercurhydrin, 39 mg Hg/ml, subcutaneously) 8 hours before \( ^{3} \text{H}-\text{NE} \) administration. These conditions did not significantly increase the 5-minute \( ^{3} \text{H}-\text{NE} \) retention in control of isoproterenol-treated rat hearts, but did abolish differences in endogenous NE content and \( ^{3} \text{H}-\text{NE} \) retention measured at 12 hours between control and isoproterenol-treated rat hearts (Table 3).

Discussion

Cardiac NE is stored in nerve terminals in dense core vesicles, and these vesicles can be removed as a part of the microsomal fraction of cardiac homogenates. Rats made hypertensive by unilateral nephrectomy and administration of DOCA and NaCl and rats subjected to repeated large doses of isoproterenol have a similar defect in storage of NE—a more rapid initial loss of \( ^{3} \text{H}-\text{NE} \) from the microsomal fraction. Since the intraneuronal NE occurs in the microsomal fraction, and since the greatest loss of \( ^{3} \text{H}-\text{NE} \) is from this fraction, presumably the inability to retain \( ^{3} \text{H}-\text{NE} \) in hearts of rats treated with isoproterenol is due to changes in the granule storage sites. Thus in both DOCA and NaCl hypertension and isoproterenol-treated rats, the cardiac storage defects seem to be intravesicular in location. Isoproterenol does not cross the nerve cell membrane, and therefore it is unlikely that it releases NE by competition for storage sites. Moreover, even at the large doses of isoproterenol used in this study, no isoproterenol or its 3-methoxy
TABLE 1
Subcellular Distribution of NE and 3H-NE in Hearts of Control and Isoproterenol-Treated Rats

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Control</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3H-NE (counts/min × 10³)</td>
<td>Control</td>
<td>22.3 ± 1.4</td>
<td>19.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>25.8 ± 2.7</td>
<td>366 ± 5</td>
</tr>
<tr>
<td>Specific activity (muc/µg)</td>
<td>Control</td>
<td>315 ± 22</td>
<td>351 ± 10</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>243 ± 51</td>
<td>132 ± 0.01</td>
</tr>
<tr>
<td>S/G</td>
<td></td>
<td>1.32 ± 0.04</td>
<td>1.32 ± 0.04</td>
</tr>
<tr>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3H-NE (counts/min × 10³)</td>
<td>Control</td>
<td>12.3 ± 0.3</td>
<td>9.8 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>366 ± 7</td>
<td>168 ± 7</td>
</tr>
<tr>
<td>Specific activity (muc/µg)</td>
<td>Control</td>
<td>176 ± 2</td>
<td>207 ± 1</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>168 ± 7</td>
<td>169 ± 7</td>
</tr>
<tr>
<td>Endogenous NE (µg/heart)</td>
<td>Control</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>0.27 ± 0.01</td>
<td>0.33 ± 0.01*</td>
</tr>
</tbody>
</table>

*P<0.05;  fP<0.01.

Sixteen hours after the last injection of saline or isoproterenol (see Methods), rats received 10 µc 3H-NE (9.2 c/mc) iv and were killed 5 minutes or 4 hours later, and the NE and 3H-NE of cardiac subcellular fractions were determined. S/G denotes the ratio between the supernatant and granule or microsomal fraction. Each mean ± SEM was calculated from six rat hearts.

TABLE 2
Reversibility of Isoproterenol-Induced 3H-NE Storage Defect

<table>
<thead>
<tr>
<th>Group</th>
<th>Chloroformine (IV mg/kg)</th>
<th>Days after treatment with saline or isoproterenol</th>
<th>Endogenous NE (µg/heart)</th>
<th>3H-NE Retention (muc/µg NE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>1</td>
<td>0.02 ± 0.04</td>
<td>101 ± 10</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>-</td>
<td>1</td>
<td>0.45 ± 0.03</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>1</td>
<td>0.70 ± 0.04</td>
<td>155 ± 10</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>+</td>
<td>1</td>
<td>0.50 ± 0.02</td>
<td>128 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>1</td>
<td>0.50 ± 0.06</td>
<td>141 ± 18</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>-</td>
<td>7</td>
<td>0.72 ± 0.04</td>
<td>173 ± 25</td>
</tr>
</tbody>
</table>

Sixteen hours (1 day) after the last injection of saline or isoproterenol (see Methods), four groups of rats received 10 µc 3H-NE (9.2 c/mc) iv. Fifteen minutes after 3H-NE, seven control and seven isoproterenol-treated rats were given 10 mg/kg chloroformine-HCl subcutaneously. Two similar groups received the same dose of 3H-NE seven days after saline or isoproterenol treatment was completed. All rats were killed 8 hours after receiving 3H-NE. Mean ± SEM were computed. The significance of difference between values of isoproterenol-treated and the corresponding control is indicated. *P<0.01;  fP<0.05.

metabolite is detectable 12 hours after its administration. Thus it appears that isoproterenol disturbs the intraneural-granular NE storage sites indirectly from some extraneural location.

An acute increase in heart weight can be associated with an alteration of tissue electrolytes (20). Isoproterenol administration results in focal microscopic areas of myocardial necrosis (1, 2), but areas adjacent to focal necrosis have an alteration in their electrolyte composition resembling that of hypertrophied hearts (21). Isoproterenol produces an increase in myocardial content of sodium without altering the potassium concentration (2, 6). With DOCA and NaCl treatment, elevation in both ions has been observed (7). Although the level of total tissue...
TABLE 3
Effect of Sodium Restriction and a Mercurial Diuretic on $^3$H-NE Retention

<table>
<thead>
<tr>
<th>Group</th>
<th>Na restriction</th>
<th>ISOPROTERENOL</th>
<th>ENDOGENOUS NE (pg/mg)</th>
<th>5 min</th>
<th>12 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>+</td>
<td>0.69 ± 0.05</td>
<td>147 ± 13</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>-</td>
<td>-</td>
<td>0.66* ± 0.05</td>
<td>159 ± 18</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>-</td>
<td>0.64 ± 0.03</td>
<td>167 ± 7</td>
<td>54 ± 6</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>+</td>
<td>+</td>
<td>0.69 ± 0.06</td>
<td>178 ± 11</td>
<td>49 ± 6</td>
</tr>
</tbody>
</table>

Animals received a normal diet and tap water during the first 5 days of isoproterenol administration (see Methods), but during the fourth day a sodium-free diet and 5% sucrose in distilled water was substituted in the indicated groups. Eight hours before administration of $^3$H-NE, these two groups also received 0.1 ml of mercuhydri subcutaneously. Animals were killed 5 minutes (five animals per group) or 12 hours (seven animals per group) after receiving 5 µc of $^3$H-NE (9.2 Ci/mg). Cardiac $^3$H-N.E. and NE were determined and results computed as the mean ± S.E.

*P < 0.01; tP < 0.001.

electrolytes may be misleading, the similarity of the total tissue electrolyte changes in isoproterenol-treated and DOCA and NaCl hypertensive rat hearts and the similarity of their amine storage defects indicate that such association is possible. The reversal of the NE storage defect of isoproterenol-treated rats by a mercurial diuretic resembles the correction seen in DOCA and NaCl hypertensive rats when placed on a low-salt diet (5). The restoration of normal NE storage after a mercurial diuretic is probably secondary to an alteration of the electrolytes in the nerve terminal, as a consequence of the primary renal effect of the mercurial diuretic (22). Mercurial diuretics have a gradual prolonged action in rats, and thus changes in blood volume and cardiac output secondary to diuretics would probably develop gradually. If acute circulatory changes were significant, they would decrease, not increase, the retention of $^3$H-NE. Proof of the presence of an altered electrolyte composition in the cardiac sympathetic nerve terminals must await the development of new techniques.

Although the fraction of the cardiac output delivered to the coronary arteries is not abnormal 16 hours after the last dose of isoproterenol, if the cardiac output were decreased, the coronary perfusion and oxygen supply might be below myocardial requirements. Thus, an increase in the concentration of acid metabolites, induced by hypoxia, could release NE from local nerve terminals (33). This local effect, combined with increased sympathetic nerve activity secondary to a change in baroreceptor stimulation, could produce an accelerated loss of neuronal NE. The increased utilization of NE would result in a fall in NE levels if synthesis failed to keep pace with release. Since chlorisondamine can abolish the cardiac $^3$H-NE storage defect in both DOCA and NaCl hypertension and isoproterenol-treated rat hearts, an increase in sympathetic activity may be present in both conditions. The ability of a diuretic and a ganglionic blocking agent to so readily reverse the NE storage defect in isoproterenol-treated rat hearts would imply the defect is not due simply to widespread necrosis of myocardial nerve endings. Preliminary results indicate that the total cardiac tyrosine hydroxylase activity, which is confined to sympathetic nerves in peripheral tissues (24), is not altered by the dosage schedule of isoproterenol used here. Moreover, the ability of the nerve endings to store NE returns to normal after the administration of isoproterenol is stopped, which would also make nerve necrosis an unlikely explanation of the NE storage defect. The presence or absence of degenerative or destructive changes in cardiac sympathetic nerve endings after isoproterenol treatment must await electron photomicrographic studies.
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The defect in \(^{3}H\)-NE storage described here may indicate the accelerated release of endogenous NE from the heart. Similarly, the numerous arrhythmias associated with coronary occlusion or cardiac hypertrophy of other causes may be related to increased release of endogenous NE secondary to alterations in tissue electrolytes. This hypothesis would explain the increased mortality of isoproterenol-treated animals that received high-sodium, low-potassium diets, or mineralocorticoids in addition to isoproterenol (24, 25).

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