Influence of Pituitary, Thyroid, and Adrenal Hormones on Norepinephrine Turnover and Metabolism in the Rat Heart

By Lewis Landsberg, M.D., and Julius Axelrod, Ph.D.

ABSTRACT

Norepinephrine turnover was estimated in the rat heart from the rate of release of tracer $^3$H-norepinephrine and the depletion of endogenous norepinephrine after inhibition of synthesis. Cardiac norepinephrine turnover was markedly increased in chronically hypophysectomized rats. Thyroidectomy and adrenalectomy were also associated with increased cardiac norepinephrine turnover, whereas ovariectomy was not. Chronic treatment of hypophysectomized rats with thyroxine and ACTH restored the turnover to normal, the replacement of thyroxine being quantitatively more significant.

Cardiac atrophy and decreased blood pressure occurred concomitantly with increased norepinephrine turnover in the hypophysectomized rat and were restored to normal with thyroid treatment. Ganglionic blockade of the hypophysectomized rat also restored the turnover toward normal. The metabolites of $^3$H-norepinephrine were shifted with a decrease in O-methylated metabolites and an increase in deaminated products.

This evidence suggests that thyroid deficiency and, to a lesser extent, adrenal deficiency are associated with an increase in cardiac norepinephrine turnover mediated by an increase in sympathetic nervous activity. The role of the increased turnover in relation to the hormonal deficiencies is discussed.

ADDITIONAL KEY WORDS $\alpha$-methyl-para-tyrosine ACTH thyroidectomy thyroxine norepinephrine synthesis ovariectomy adrenalectomy $^3$H-norepinephrine isolated perfused heart ganglionic blockade $^3$H-norepinephrine metabolites

Norepinephrine, the adrenergic neurotransmitter, is synthesized in the postganglionic sympathetic nerve fibers (1) and stored there in dense core vesicles (2, 3). It is released in response to sympathetic nerve impulses (4-6). A tracer dose of $^3$H-norepinephrine, after intravenous administration, is taken up by the axonal membrane and concentrated in the sympathetic nerve ending (7, 8), where it equilibrates with the endogenous norepinephrine stores (9, 10).

Previous studies on the disposition of $^3$H-norepinephrine in the hypophysectomized rat have shown that its retention is decreased in the heart (11). This finding suggested that the turnover of cardiac norepinephrine was increased. In the present study, the turnover of norepinephrine in the hearts of hypophysectomized rats was determined by measuring the rate of decrease (a) of specific activity of cardiac norepinephrine after the administration of $^3$H-norepinephrine tracer (9) and (b) of endogenous norepinephrine after the administration of alpha-methyl-para-tyrosine, an inhibitor of norepinephrine synthesis (12).

Both of these methods showed that cardiac norepinephrine turnover was increased following hypophysectomy. Ablation or replacement experiments were also performed to delineate the specific hormonal deficiencies that produce the increased cardiac norepinephrine turnover in hypophysectomized rats. The results indicate that both thyroid and adrenal deficiency are associated with increased cardiac norepinephrine turnover, but the effects of thyroid are quantitatively more important.
**Methods**

Female Sprague-Dawley rats were used in all the experiments. Surgical ablation of the hypophysis, thyroid, adrenals, and ovaries was performed by Hormone Assay Laboratories, Inc., Chicago, Illinois. All animals were operated on when they weighed 180 g, and they were maintained at least 1 month after operation on normal laboratory diet fortified with oranges, bread, and milk. The adrenalectomized animals, in addition, were allowed free access to isotonic saline as well as to water. Paired, age-matched controls had no operations or sham hypophysectomies. The adequacy of surgical ablation was verified for each animal by direct inspection of the appropriate areas at autopsy and by the expected changes in organ and body weight.

\[ d^{-7}{\text{H}}\text{-norepinephrine (specific activity, 5 to 10 c/mmole) was obtained from New England Nuclear Corporation. It was purified before use by adsorption onto an alumina column at pH 8.6 and eluted with 0.2 N acetic acid. The tracer was administered to unanesthetized animals via the tail vein after appropriate dilution with isotonic saline. The methyl ester of alpha-methyl-para-tyrosine (Hassle 44/68) was dissolved in saline and administered intraperitoneally. Chlorisondamine (Ecolid, Ciba) was administered in isotonic saline in doses of 15 mg/kg ip. Sodium levothyroxine was injected subcutaneously, 10 μg/day in dilute alkaline aqueous solution. Purified corticotropin (ACTH) in gelatin (Cortrophin Gel, Organon) was injected subcutaneously in doses of 4 units per day. Adequacy of hormonal treatment was judged by increased heart weight in the thyroid-treated hypophysectomized rats (13) and increased adrenal weight in the rats treated with ACTH. Systolic blood pressure was taken in the tail artery of warmed, unanesthetized rats with a pulse transducer applied to the tail (14). The animals were killed by cervical dislocation, and the organs were rapidly removed, weighed, and homogenized in ice cold 0.4 N perchloric acid. Norepinephrine was isolated from the perchloric acid extract by adsorption on alumina at pH 8.6 (15) and eluted with 0.2 N acetic acid. Aliquots of the alumina eluate were counted for \[^3\text{H}\]norepinephrine by liquid scintillation spectrometry at an efficiency of about 10% for tritium. Endogenous norepinephrine was determined on the alumina eluate spectrofluorometrically by the trihydroxyindole method of Euler and Lisjak (16). Values were corrected for a recovery of 80 to 90% as determined for each experiment. Adrenal catecholamines were determined by the trihydroxyindole method on the perchloric acid extract of adrenal glands after a fifty-fold dilution.

\[^3\text{H}\]metabolites of \[^3\text{H}\]norepinephrine were determined as previously described (17), both in vivo and in the isolated perfused heart. The isolated heart was perfused by the Langendorff technique with oxygenated Krebs-Henseleit solution at 37°C at a \[^3\text{H}\]norepinephrine concentration of 5 ng/ml (3 × 10^{-8} M). The perfusion was maintained for 10 minutes at a constant flow of 3.5 ml/min by a Harvard pump and was followed by a 2-minute wash without \[^3\text{H}\]norepinephrine. The total uptake of \[^3\text{H}\]norepinephrine was considered the \[^3\text{H}\]norepinephrine recovered from the heart at the end of the perfusion plus the sum of the metabolites in the heart, perfusate, and wash. Total metabolites present in the heart, perfusate and wash were expressed as percent of total \[^3\text{H}\]norepinephrine uptake.

In the studies of norepinephrine turnover, the data are plotted semilogarithmically according to the method of least squares weighted for the number of animals and standard error. The slope...
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1. Turnover of Cardiac Norepinephrine Following Hypophysectomy.—As previously described (11), the retention of a tracer dose of 3H-norepinephrine in the hearts of hypophysectomized rats was markedly impaired. This finding was not due to impaired uptake or delivery of tracer, since the initial concentration of 3H-norepinephrine in the heart was normal (Fig. 1). The decrease in specific activity of cardiac norepinephrine at various times after the administration of a tracer dose of 3H-norepinephrine is shown in Figure 2. The turnover of cardiac norepinephrine in the hypophysectomized rats was about twice that in the control rats. The synthesis of norepinephrine, calculated from the slope of the regression line and endogenous norepinephrine content, was 180.6 ng/g/hour for the hypophysectomized rats and 72.8 ng/g/hour for the controls.

The endogenous norepinephrine concentration, heart weight and blood pressure are given in Table 1. The endogenous norepinephrine did not change over the course of the experiment. The blood pressure of hypophysectomized rats was regularly and significantly reduced as compared with normal or controls with sham operations.

Norepinephrine turnover in the heart was also measured by the decrease in endogenous norepinephrine after synthesis had been inhibited with alpha-methyl-para-tyrosine (Fig. 3). The half-time determined by this method was 5.1 hours for the hypophysectomized rats.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat wt (g)</th>
<th>Heart wt (g)</th>
<th>Norepinephrine (ug/heart)</th>
<th>Systolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>218.9 ± 1.8</td>
<td>0.699 ± 0.011</td>
<td>0.781 ± 0.026</td>
<td>1.12 ± 0.04</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>160.7* ± 1.3</td>
<td>0.417* ± 0.003</td>
<td>0.618* ± 0.024</td>
<td>1.48* ± 0.06</td>
</tr>
</tbody>
</table>

All values are means ± SEM. BP = blood pressure for 6 rats of each group; other data are for 19 rats. Details as described for Figure 2.

*P < 0.001.
TABLE 2

Endogenous Norepinephrine of Brain, Spleen, and Salivary Glands after Inhibition of Synthesis with Alpha-Methyl-Para-Tyrosine

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt (g)</th>
<th>Control (μg/g)</th>
<th>3.5 Hours after αMT*</th>
<th>Untreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>1.763</td>
<td>0.376</td>
<td>0.209†</td>
<td>55.6†</td>
</tr>
<tr>
<td></td>
<td>±0.025</td>
<td>±0.020</td>
<td>±0.011</td>
<td>±2.9</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>1.748</td>
<td>0.402</td>
<td>0.202†</td>
<td>50.2†</td>
</tr>
<tr>
<td></td>
<td>±0.21</td>
<td>±0.020</td>
<td>±0.012</td>
<td>±3.0</td>
</tr>
<tr>
<td><strong>Spleen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>0.708</td>
<td>1.19</td>
<td>0.87‡</td>
<td>72.3‡</td>
</tr>
<tr>
<td></td>
<td>±0.027</td>
<td>±0.08</td>
<td>±0.09</td>
<td>±7.9</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>0.398</td>
<td>1.54</td>
<td>0.79†</td>
<td>51.0†</td>
</tr>
<tr>
<td></td>
<td>±0.018</td>
<td>±0.21</td>
<td>±0.02</td>
<td>±1.4</td>
</tr>
<tr>
<td><strong>Salivary Glands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>0.492</td>
<td>1.42</td>
<td>0.86§</td>
<td>60.6§</td>
</tr>
<tr>
<td></td>
<td>±0.011</td>
<td>±0.12</td>
<td>±0.07</td>
<td>±5.1</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>0.265</td>
<td>1.76</td>
<td>1.23</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>±0.008</td>
<td>±0.17</td>
<td>±0.17</td>
<td>±9.6</td>
</tr>
</tbody>
</table>

*Alpha-methyl-para-tyrosine; †P < 0.001; ‡P < 0.05; §P < 0.01, for difference from untreated controls. Experimental details are as outlined in Figure 3; there were 3 to 5 rats in each group.

TABLE 3

Adrenal Catecholamine Content after Inhibition of Synthesis with Alpha-Methyl-Para-Tyrosine

<table>
<thead>
<tr>
<th>Adrenal wt (g/pair)</th>
<th>Control*</th>
<th>3.5 Hours after αMT*</th>
<th>6 Hours after αMT*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>NE</td>
<td>E + NE</td>
</tr>
<tr>
<td><strong>Sham Operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.082</td>
<td>39.1</td>
<td>8.0</td>
<td>47.2</td>
</tr>
<tr>
<td>±0.003</td>
<td>±4.1</td>
<td>±3.6</td>
<td>±3.1</td>
</tr>
<tr>
<td><strong>Hypophysectomy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>23.3</td>
<td>5.4</td>
<td>28.6</td>
</tr>
<tr>
<td>±0.002</td>
<td>±1.3</td>
<td>±1.8</td>
<td>±1.4</td>
</tr>
</tbody>
</table>

*Values are μg/pair of adrenals. E = epinephrine; NE = norepinephrine; αMT = alpha-methyl-para-tyrosine. Experimental details are as described in Figure 3; there were 5 rats in each group.

and 18.1 hours for the controls with sham operations; corresponding calculated synthesis rates were 172.7 and 42.6 ng/g/hour, respectively. Analyses of spleen, salivary gland, brain, and adrenals for endogenous catecholamines were also performed on the animals treated with alpha-methyl-para-tyrosine, and the results are summarized in Tables 2 and 3. In the spleens of hypophysectomized rats, the reduction in norepinephrine was slightly greater than in the controls with sham operations, suggesting increased turnover; but in the brain, salivary glands, and adrenals the reduction produced by alpha-methyl-para-tyrosine was the same in both groups.

2. Effect of Ganglionic Blockade on Cardiac Norepinephrine Turnover in Hypophysectomized Rats.—To examine the influence of centrally mediated sympathetic activity on the increased turnover of cardiac norepinephrine in the hypophysectomized rat, the effect of decentralization by chlorisondamine was studied. Ganglionic blockade increased the retention of 3H-norepinephrine more in the hearts...
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FIGURE 3

Turnover of cardiac norepinephrine following hypophysectomy; rate of depletion after synthesis inhibition. Sixty-seven days after hypophysectomy or sham hypophysectomy, rats were divided into 3 groups: untreated controls received saline; rats in the second group received alpha-methyl-para-tyrosine, 250 mg/kg, and were killed 3½ hours later; rats in the third group received 250 mg/kg of the same drug followed by 125 mg/kg 3 hours later and were killed 6 hours after the first dose. Each point represents the mean ± SEM for 4 or 5 animals. Slope for hypophysectomized rats = \(-0.1365 ± 0.02068\); for those with sham operations = \(-0.0382 ± 0.01474\). Blood pressure before study was 84.5 ± 4.1 mm Hg for hypophysectomized rats and 119.9 ± 3.9 for those with sham operations (P < 0.001).

FIGURE 4

Effect of ganglionic blockade on norepinephrine turnover in hypophysectomized rats. Eighty days after hypophysectomy or sham hypophysectomy, rats were injected with \(^3\)H-norepinephrine, 25 \(\mu\)g/kg. One-half the animals of each group received chlorisondamine, 15 mg/kg, 5 minutes after the \(^3\)H-norepinephrine was administered. All were killed 6 hours later. Each bar represents the mean ± SEM for 8 or 9 animals, in counts/min/g. **P < 0.01 for difference between untreated hypophysectomized rats and untreated rats with sham operations; no significant difference between either chlorisondamine-treated group. Percentage over bar is the increase over the group without ganglionic blockade.

of hypophysectomized rats than in the hearts of controls with sham operations. Six hours after the administration of \(^3\)H-norepinephrine, the untreated hypophysectomized rats had lower \(^3\)H-norepinephrine levels in the heart than the untreated rats with sham operations, consistent with increased turnover; the rats treated with chlorisondamine, however, showed no difference between the control and hypophysectomized rats at 6 hours (Fig. 4). Ganglionic blockade was associated with a greater increase in \(^3\)H-norepinephrine retention in the hypophysectomized rats than in the controls. Since ganglionic blockade caused
TABLE 4
Uptake and Metabolism of \(^{3}H\)-Norepinephrine by Isolated Perfused Hearts of Hypophysectomized Rats

<table>
<thead>
<tr>
<th>Heart wt (g)</th>
<th>(^{3}H)-norepinephrine uptake (cpm X 10^3/heart)</th>
<th>Deaminated (^{3}H)-metabolites (cpm X 10^3/heart)</th>
<th>% of NE uptake</th>
<th>(^{3}H)-normetanephrine (cpm X 10^3/heart)</th>
<th>% of NE uptake</th>
<th>Total (^{3}H)-deaminated O-methylated metabolites (cpm X 10^3/heart)</th>
<th>% of NE uptake</th>
<th>Metabolite total (cpm X 10^3/heart)</th>
<th>% of NE uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.888 ± 0.026</td>
<td>0.532* ± 0.028</td>
<td>2.0 ± 0.6</td>
<td>5.36 ± 0.5</td>
<td>2.5 ± 0.3</td>
<td>1.10 ± 0.3</td>
<td>2.0 ± 0.5</td>
<td>6.9 ± 0.9</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>3.84* ± 0.026</td>
<td>12.44* ± 0.026</td>
<td>3.84 ± 0.04</td>
<td>19.2 ± 0.2</td>
<td>3.4 ± 0.1</td>
<td>4.27 ± 0.4</td>
<td>3.9 ± 0.04</td>
<td>18.9 ± 0.1</td>
<td>3.9 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

The deaminated metabolites (3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylglycol) were present in the heart, perfusate, and wash; highest concentration was in the perfusate. Normetanephrine was present almost exclusively in the heart. The deaminated-O-methylated products (3-methoxy-4-hydroxymandelic acid and correspond- ing glycol) were present almost exclusively in the perfusate.

*P < 0.001; †P < 0.01; ‡P < 0.05, for difference from control rats.

3. Metabolism of \(^{3}H\)-Norepinephrine by the Hearts of Hypophysectomized Rats.—The uptake of \(^{3}H\)-norepinephrine in isolated hearts perfused by the Langendorff technique was unimpaired (Table 4) and, per gram of heart, was actually increased after hypophysectomy. There was a shift in the pattern of metabolites: the hypophysectomized rats produced less of the O-methylated metabolite (normetanephrine) and more deaminated catechols. In vivo (Table 5), 5 minutes after administration of \(^{3}H\)-norepinephrine, the uptake was also unimpaired and the production of normetanephrine was significantly reduced, but there was no change in deaminated catechols. Preliminary studies showed no changes in monoamine oxidase or catechol-O-methyltransferase activity in the heart that would account for these results.

TABLE 5
In Vivo Uptake and Metabolism of \(^{3}H\)-Norepinephrine in Hearts of Hypophysectomized Rats

<table>
<thead>
<tr>
<th>Heart wt (g)</th>
<th>(^{3}H)-norepinephrine (cpm X 10^3/heart)</th>
<th>Deaminated (^{3}H)-metabolites (cpm X 10^3/heart)</th>
<th>% T.C.</th>
<th>(^{3}H)-normetanephrine (cpm X 10^3/heart)</th>
<th>% T.C.</th>
<th>Total (^{3}H)-deaminated O-methylated metabolites (cpm X 10^3/heart)</th>
<th>% T.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Operation</td>
<td>0.915 ± 0.029</td>
<td>127.0 ± 0.9</td>
<td>1.86</td>
<td>15.7 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.12 ± 0.3</td>
<td>1.0 ± 0.04</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>0.581* ± 0.014</td>
<td>104.1 ± 8.3</td>
<td>1.07 ± 0.14</td>
<td>5.51* ± 0.95</td>
<td>1.08</td>
<td>1.74 ± 0.14</td>
<td>1.29 ± 1.02</td>
</tr>
</tbody>
</table>

Hypophysectomized rats and rats with sham operations (68 days after operation) were injected with 100 \(\mu\)c/kg \(^{3}H\)-norepinephrine and killed 5 minutes later. Hearts were assayed for metabolites as described under Methods. Total counts (T.C.) refer to radioactivity of perchlorate extract. Hypophysectomized rats weighed 191.8 ± 3.8 g; (mean dose was 19.1 \(\mu\)c). Rats with sham operations weighed 271.7 ± 4.6 g; (mean dose was 27.2 \(\mu\)c). There were 6 or 7 animals in each group. Results are expressed as mean ± SEM.

*P < 0.001; †P < 0.05, for difference from controls.

lethargy and dystonic posturing in the hypophysectomized rats, the experiment was ended at 6 hours. It is likely, however, that the difference described here would have been accentuated by more time.

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4. Effect of Thyroid, Adrenal, and Ovarian Ablation on Norepinephrine Turnover in the Heart.—Twenty-four hours after administration of a tracer dose of $^3$H-norepinephrine, the specific activity of cardiac norepinephrine was reduced in the thyroidectomized and adrenalectomized rats, but in the ovariec-
tomized rats it increased slightly (Fig. 5). Organ weight and endogenous norepinephrine are presented in Table 6. The uptake of $^3$H-norepinephrine in the heart at 5 minutes was the same in thyroidectomized, adrenalecto-
mized, and untreated rats. This indicates that the reduced retention of $^3$H-norepinephrine is due to increased norepinephrine turnover.

5. Cardiac Norepinephrine Turnover in Hypophysectomized Rats Treated with Replacement Doses of Thyroxine and ACTH.—The evidence from the ablation studies suggested that thyroid and adrenal deficiency were responsible for the increased turnover of cardiac norepinephrine in hypophysectomized rats. This was confirmed by treatment of chronically hypophysectomized animals with replacement doses of thyroxine and ACTH for 3 weeks. ACTH was selected rather than an adrenal steroid because of the availability of a preparation of known potency and because replacement of a single adrenal steroid might not adequately replace the spectrum of biologic effects of adrenal secretion. When considered with the effect of adrenalectomy, it seems likely that the effect of ACTH described here is secondary to its trophic effect.

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TABLE 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat wt (g)</th>
<th>Heart wt (g)</th>
<th>Endogenous norepinephrine (µg/heart)</th>
<th>Endogenous norepinephrine (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>233.1</td>
<td>0.781</td>
<td>0.954</td>
<td>1.22</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>180.7*</td>
<td>0.536*</td>
<td>0.692†</td>
<td>1.29</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>221.0‡</td>
<td>0.798</td>
<td>0.977</td>
<td>1.21</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>276.0*</td>
<td>0.894†</td>
<td>1.01</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM; 6 or 7 animals in each group.

$^*P<0.001$; $†P<0.01$; $‡P<0.05$, for difference from control.

Circulation Research, Vol. XXII, May 1968
Treatment of hypophysectomized rats with replacement doses of thyroxine and ACTH. Hypophysectomy or sham hypophysectomy was performed on 180-g rats. The rats with sham operations and some of the hypophysectomized rats received no hormonal replacement. Beginning 21 days after the operation, other hypophysectomized rats received daily injections of thyroxine (T₄), ACTH, or thyroxine and ACTH for the next 3 weeks.

on the adrenal cortex rather than a direct action. Thyroxine, on the other hand, is known to replace all the physiologic effects of thyroid secretion. The plan for the replacement experiment is described in the legend to Figure 6. The retention of a tracer dose of ^3H-norepinephrine by each of the treated groups is shown in Figure 7. Significant increases in ^3H-norepinephrine retention were produced by treatment with thyroxine alone or ACTH alone, but complete restoration to normal required replacement of both hormones (Fig. 7). Further evidence that the increased ^3H-norepinephrine retention in the hormone-treated group represents decreased turnover is shown by an experiment in which norepinephrine synthesis was inhibited. After treatment with alpha-methyl-para-tyrosine there was a much greater decrease in endogenous norepinephrine in the untreated hypophysectomized group than in the controls with sham operations or the hypophysectomized group treated with thyroxine and ACTH (Fig. 8).

Organ weights and blood pressure for all the treatment groups are presented in Table 7. Appropriate changes in heart and adrenal weight confirm the efficacy of the hormone treatment. Treatment with ACTH alone had no effect on blood pressure, but thyroxine alone or in combination with ACTH restored it to normal. In Table 8 the endogenous norepinephrine and ^3H-norepinephrine levels after tracer administration are given. It is of interest that blood pressure and heart weight

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Effect of Thyroxine and ACTH Treatment on Organ Weight and Blood Pressure in Chronically Hypophysectomized Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Treatment</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>ACTH</td>
</tr>
<tr>
<td></td>
<td>thyroxine</td>
</tr>
<tr>
<td></td>
<td>ACTH +</td>
</tr>
<tr>
<td></td>
<td>thyroxine</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM.

*Over the course of the experiment once replacement treatment had begun. †P < 0.001; ‡P < 0.05; §P < 0.01, for difference from untreated hypophysectomized rats.
are inversely related to norepinephrine turnover (Fig. 9).

**Discussion**

The results described clearly demonstrate an increased turnover of cardiac norepinephrine in hypophysectomized rats. The ablation and replacement experiments indicate that thyroid and, to a lesser extent, adrenal deficiency produce this increase in turnover. In the presence of normal levels of endogenous norepinephrine, an increase in norepinephrine turnover implies increased synthesis. The synthesis rates calculated from turnover studies and endogenous norepinephrine levels, suggest about a threefold increase in norepinephrine synthesis in the hearts of hypophysectomized rats. The more rapid depletion in cardiac endogenous norepinephrine in hypophysectomized rats following inhibition of tyrosine hydroxylase, together with the fact

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*Figure 7*

Effect of thyroxine and ACTH replacement on the retention of \(^{3}H\)-norepinephrine in the heart of hypophysectomized rats. All animals received \(^{3}H\)-norepinephrine, 20 \(\mu\)g/kg and were killed 24 hours later. Each bar represents the mean ± SEM for 7 to 9 animals. \(*P < 0.05; \***P < 0.001\) for difference from untreated rats with sham operations. Significant difference \((P < 0.001)\) also existed between the untreated hypophysectomized rats and all the treated groups of hypophysectomized rats. The significance of the difference between the ACTH-treated rats and the thyroxine-treated rats was \(P < 0.001\) and for the thyroxine-treated rats and those treated with both drugs was \(P < 0.05\). See legend to Figure 6 for description of experiment.

*Figure 8*

Effect of thyroxine and ACTH replacement on cardiac norepinephrine depletion after inhibition of synthesis. Hypophysectomized rats, rats with sham hypophysectomy, and hypophysectomized rats receiving ACTH and thyroxine replacement were given alpha-methyl-\(p\)-tyrosine, 250 mg/kg, and killed after 4 hours. Results are expressed as the mean percent (±SEM) reduction in endogenous norepinephrine as compared with similar groups of animals not receiving alpha-methyl-\(p\)-tyrosine. (Control norepinephrine values are given in Table 8.) The actual norepinephrine values for these animals (5 or 6 in each group) in \(\mu\)g/g were: with hypophysectomy, 0.780 ± 0.098; with sham operations, 1.009 ± 0.064; with hypophysectomy plus ACTH and thyroxine, 1.097 ± 0.077.
that tyrosine hydroxylation is the rate-limiting step in norepinephrine biosynthesis in the heart (12), suggests that it is the hydroxylation of tyrosine that is increased after hypophysectomy. To establish this, further studies involving the rate of incorporation of precursors into cardiac norepinephrine will be required.

The mechanisms involved in the regulation of norepinephrine biosynthesis are incompletely understood, but it seems likely that feedback inhibition of tyrosine hydroxylase by

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catechols is involved (18). Thyroxine is itself a tyrosine derivative and moniodotyrosine and diiodotyrosine are potent inhibitors of this enzyme (18, 19). Since thyroxine administration reduced the turnover (and presumably the synthesis) of norepinephrine in these thyroid-deficient rats, it is possible that the effect of this hormone might be on tyrosine hydroxylase itself.

The increased turnover of norepinephrine demonstrated here is limited to the hearts, and perhaps the spleens of hypophysectomized rats. There is no evidence of a generalized increase in sympathetic activity such as occurs in vigorous muscular exercise (20). The restoration of cardiac norepinephrine turnover to normal after ganglionic blockade implicates centrally mediated sympathetic activity in the genesis of the increased turnover.

The significance of increased cardiac norepinephrine turnover in the hypophysectomized rat is unknown. The inverse relationship (Fig. 9) between blood pressure and cardiac norepinephrine turnover suggests that the increased turnover is compensatory. Cardiac atrophy after hypophysectomy or thyroidectomy is marked (Tables 1 and 6) (13) and the cardiac output is known to be low in these states (21-24). Recent studies indicate a direct effect of thyroid hormone on myocardial contractility (25). Furthermore, the decreased cardiac output in hypophysectomized rats is restored to normal by thyroxine treatment (22, 26). If cardiac atrophy and decreased contractility are associated with circulatory insufficiency, then an increase in adrenergic activity with improvement in myocardial performance might be anticipated. Such an increase in adrenergic activity is noted in congestive heart failure (27). However the available hemodynamic data in hypophysectomized rats and hypothyroid subjects does not fully support this analogy (22). Hypophysectomized rats (21) and hypothyroid patients (23) are capable of normal or near normal increases in cardiac output in response to exercise or circulatory stress, and the normal signs of circulatory insufficiency such as organ congestion and increased pulmonary and left ventricular end-diastolic pressure are absent. The available evidence seems to indicate that the circulatory changes in patients with thyroid or pituitary deficiency are secondary to decreased metabolism and oxygen consumption in the peripheral tissues. Further studies are needed to determine whether a primary hemodynamic defect exists in the absence of thyroid or adrenal secretion and whether the increased norepinephrine turnover described here is compensatory to hypotension and decreased cardiac output.

Thyroid and adrenal deficiency are known to be associated with decreased responsiveness to norepinephrine (28-30). It is not likely that decreased responsiveness would itself result in a compensatory increase in norepinephrine turnover because the denervated heart can function adequately (31, 32). In the presence of a hemodynamic defect, however, this may be a contributory factor. In view of the role of cyclic 3'5'-adenosine monophosphate (AMP) in mediating the cardiac effects of norepinephrine (33) and the fact that thyroid and adrenal hormones are required for the normal function and activation of adenylylcyclase, which produces this key intermediate (34), the status of the cyclic AMP-adenyl cyclase system in the hypophysectomized rat heart deserves investigation.

The production of normetanephrine by O-methylation is the major metabolic pathway for the inactivation of physiologically active norepinephrine (4, 35, 36). The finding of decreased normetanephrine production in the hypophysectomized rat heart, if confirmed, implies that some of the norepinephrine turnover may be intraneuronal with the production of inactive metabolites. This may indicate that the increased turnover of norepinephrine in the heart is concerned less with cardiac stimulation than with some other vital function (related, perhaps, to peripheral vasoconstriction and heat conservation).

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References


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