Catecholamine-synthesizing enzymes in the rat adrenal gland during exposure to cold

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During exposure to cold there is an increase in the excretion of urinary catecholamines (12, 25, 31). Cottle and Carlson (6) showed that adrenal demedullation only partially removes the calorogenic response to cold in acclimated rats, and it is generally accepted that norepinephrine discharged from the sympathetic neurons is mainly responsible for thermogenesis in cold-adapted rats (5). The catecholamines secreted from the adrenal medulla, however, play a significant role in maintaining the body temperature of unacclimated rats exposed to cold (26). Gordon et al. (16), however, did not find an increase in the turnover of adrenal epinephrine after short-term exposure of rats to cold temperatures. In the present study we have examined the levels of adrenal catecholamine-synthesizing enzymes after exposure of rats to cold for varying lengths of time.

METHODS

Male Sprague Dawley rats weighing 180–200 g at the start of the experiment were continuously exposed to a temperature of 3 C for 1, 7, 21, or 42 days before being killed. The animals were kept in the cold room in plastic cages that contained about 0.25 inch of sawdust and were covered with open wire-mesh tops. Since isolation is also a stress which produces changes in adrenal enzymes (unpublished observations), five animals were kept in each cage. The animals received food and water ad libitum. Control animals and experimental animals before cold exposure were kept at room temperature under the same light cycles and housing conditions as during cold exposure. All animals were decapitated 42 days after the start of the experiment, on the same day after cold exposure for the indicated number of days before the day on which they were killed. The adrenal glands were rapidly removed cleaned, weighed, and homogenized in 1.0 ml cold isotonic sucrose. An aliquot (100 µl) of the sucrose homogenate was added to 0.9 ml 0.4 N perchloric acid for assay of catecholamines (2). A second aliquot (100 µl) of the homogenate was added to 200 µl 0.15% Triton-X; 50 µl of this mixture was assayed for DBH by the method of Friedman and Kaufman (13) as modified by Viveros et al. (34). The remainder of the homogenate was centrifuged at 26,000 × g for 20 min, and aliquots of the clear supernatant were assayed for PNMT (3) and for TH (27).

A WIDE VARIETY of stresses or treatments result in release of catecholamines from the adrenal medulla. Release of epinephrine from the isolated perfused adrenal of cats, induced by splanchnic nerve stimulation or by acetylcholine administration, exceeds the amount of catecholamines depleted from this organ (4, 18). Von Euler (10) attributed this finding to rapid resynthesis of the catecholamines following its release.

In rats, during exercise there is an increase in the adrenal epinephrine turnover rate and in the synthesis of catecholamines from tyrosine-14C (16). Immobilization stress also increases the synthesis of adrenal catecholamines from tyrosine-14C in rats (unpublished observations). In addition, after repeated immobilization stress there are increases in adrenal catecholamine levels and urinary epinephrine excretion (23) and marked increases in the levels of the adrenal catecholamine-synthesizing enzymes, tyrosine hydroxylase (TH) (17, 18), phenylethanolamine-N-methyl transferase (PNMT) (22), and dopamine-β-hydroxylase (DBH) (21, 21).
TABLE 1. Effect of exposure to cold on rat adrenal weight and body weight

<table>
<thead>
<tr>
<th>Days of Exposure</th>
<th>Adrenal Weight, mg/Pair</th>
<th>Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45.82 ± 1.20</td>
<td>280.14 ± 4.71</td>
</tr>
<tr>
<td>1</td>
<td>49.35 ± 1.35</td>
<td>274.50 ± 7.27</td>
</tr>
<tr>
<td>7</td>
<td>56.17 ± 2.29*</td>
<td>286.75 ± 10.52</td>
</tr>
<tr>
<td>21</td>
<td>53.16 ± 1.33*</td>
<td>279.44 ± 11.35</td>
</tr>
<tr>
<td>42</td>
<td>54.34 ± 1.49†</td>
<td>345.50 ± 5.86†</td>
</tr>
</tbody>
</table>

Rats were continuously exposed to cold (3 C) and killed immediately after exposure. Control rats were kept at room temperature. Results are expressed as means ± SEM for groups of 7-12 rats. * P < 0.01 compared to control group. † P < 0.001 compared to control group.

TABLE 2. Effect of exposure to cold on adrenal epinephrine and norepinephrine levels

<table>
<thead>
<tr>
<th>Days of Exposure</th>
<th>Epinephrine, mg/Pair</th>
<th>Norepinephrine, mg/Pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.72 ± 2.59</td>
<td>5.48 ± 0.74</td>
</tr>
<tr>
<td>1</td>
<td>27.54 ± 1.54*</td>
<td>6.78 ± 0.58</td>
</tr>
<tr>
<td>7</td>
<td>30.27 ± 1.33</td>
<td>9.46 ± 0.93†</td>
</tr>
<tr>
<td>21</td>
<td>35.94 ± 1.74</td>
<td>11.48 ± 1.43†</td>
</tr>
<tr>
<td>42</td>
<td>31.88 ± 1.53</td>
<td>12.42 ± 1.52†</td>
</tr>
</tbody>
</table>

Rats were continuously exposed to cold (3 C) and killed immediately after exposure. Control rats were kept at room temperature. Results are expressed as means ± SEM for groups of 7-12 rats. * P < 0.05 compared to control group. † P < 0.01 compared to control group. †† P < 0.001 compared to control group.

RESULTS

Effect of exposure to cold on adrenal and body weights of rats. The adrenal weight was significantly increased after 7, 21, or 42 days of exposure to cold (Table 1). The body weight was increased over control levels only after 42 days of exposure to cold.

Effect of exposure to cold on adrenal catecholamine levels. There was a small but significant decrease in adrenal epinephrine levels after 1 day of exposure to cold (Table 2). After 7, 21, or 42 days the epinephrine levels were not different from those in control animals. Adrenal norepinephrine levels were not changed after 1 day of exposure to cold but...
were significantly elevated after 7, 21, or 42 days of exposure.

**Effect of exposure to cold on adrenal TH activity.** After 1 day of exposure to cold, adrenal TH levels were not significantly elevated; but after 7 or 21 days at 3 C, there was significant increase (Fig. 1). After 42 days, TH activity was no longer significantly increased.

**Effect of exposure to cold on adrenal DBH activity.** Levels of adrenal DBH were elevated after only 1 day of exposure to cold and were further elevated after 7 or 21 days (Fig. 2). After 42 days of exposure to cold, DBH activity was significantly lower than that found after 21 days of exposure ($P < 0.001$) but still significantly greater than that found in control animals ($P < 0.01$).

**Effect of exposure to cold on adrenal PNMT activity.** There were small but significant increases in adrenal PNMT activity after 7 or 21 days of exposure to cold (Fig. 3). PNMT levels were not significantly increased after 1 or 42 days of exposure.

**DISCUSSION**

Catecholamines secreted from the adrenal medulla play a significant role in the response to cold exposure, particularly in unacclimated rats. Maickel et al. (26) showed that adrenal-demedullated unacclimated animals were less capable of maintaining body temperature than control intact rats despite a normal pituitary-adrenocortical response, as evidenced by increased plasma corticosterone levels. The mean survival time of demedullated rats was decreased, and blood glucose levels did not increase after cold exposure. The administration of epinephrine prior to cold exposure increased the mean survival time and elevated plasma glucose levels for more than 4 hr. In rats that were demedullated and "chemically sympathectomized" (reserpine, bretylium-like agents, or ganglionic blocking agents) exposure to cold was not attended by the usual responses. There was failure to mobilize free fatty acid, no piloerection, no vasoconstriction, nor shivering. As with demedullation alone, there was decreased survival time and lack of elevation of blood glucose levels.

Des Marais and Dugal (9) reported that the adrenals of rats exposed to 0 C for 24 days contained 3 times as much epinephrine and 4 times as much norepinephrine as the adrenals of animals exposed to cold for 1 day. Comparison of these values to those in rats not exposed to cold (17) led von Euler to conclude that the results of Des Marais and Dugal (9): "... indicate that after a primary depletion of adrenaline, and possibly noradrenaline, the content of the amines in the [adrenal] gland increases gradually over the period of 24 days to figures which are higher than normal... for noradrenaline while adrenaline figures were not significantly changed (11)." Leduc (25) confirmed this interpretation of the earlier results of acute exposure to cold with regard to the fall in adrenal epinephrine but did not find a significant change in norepinephrine.

Studies of acclimation of rats to cold are usually carried out by housing animals individually in wire cages without bedding. Isolation, however, is also a stress which may result in changes in adrenal enzymes (unpublished observations). In the present study animals were kept in groups in plastic cages with bedding so that the severity of cold exposure was probably less than that used in other investigations of cold acclimation. Leduc (25) found about a 25% decrease in epinephrine content of the adrenals of 175-g rats exposed to -7 C for 24 hr, but no significant change in norepinephrine content. These findings are in agreement with the results shown in Table 2. The fall in adrenal epinephrine (Table 2) after 1 day of exposure to cold and the subsequent return to control levels as well as the rise in adrenal norepinephrine after 7 days were similar to the changes found after immobilization stress (23).

Sympathetic nerve stimulation increases the rate of norepinephrine synthesis in guinea pig vas deferens (1, 29, 30), rat heart (15), rat salivary gland (30), and cat spleen (14). After exercise (16) or immobilization (unpublished observations) there is an increase in the conversion of tyrosine-14C to catecholamines in the adrenal medulla. Treatment of rats with reserpine ("chemical sympathectomy") causes an increase in adrenal medullary TH that is dependent on an intact neuronal system (33). After immobilization stress there are neurally dependent increases in the adrenal levels of TH (21, 22, 24) and DBH (21, 24). The increase in PNMT after immobilization is not dependent on neuronal stimuli but is a consequence of enhanced secretion of adrenal corticoids (21, 22, 24). The increases in adrenal levels of TH (Fig. 1) and DBH (Fig. 2) after exposure to cold are, therefore, probably dependent on an intact neuronal system, whereas the increase in PNMT levels (Fig. 3) is probably mediated hormonally. H. Thoenen (personal communication) has also found an increase in TH activity after 1 week of exposure to cold, but this increase was blocked by adrenal denervation.

Jansky et al. (20) examined the calorigenic effects of epinephrine and norepinephrine in rats which were acclimated to varying degrees of cold for at least 3 weeks. They suggested that thermogenesis due to adrenaline comes into action during acute stress only, whereas thermogenesis due to noradrenaline participates in nonshivering thermogenesis developing as a result of a prolonged stay in the cold.

After prolonged exposure to cold (49 days) the levels of adrenal TH (Fig. 1) and DBH (Fig. 2) were lower than those found after 21 days of exposure. The increase in PNMT levels after cold exposure (Fig. 3) was less than that seen in TH or DBH. After 42 days the PNMT levels were no longer significantly different from those in control rats. The lower activities of the synthetic enzymes found after 42 days of exposure to cold correlate well with the decrease in urinary excretion of both norepinephrine and epinephrine (Table 2) after 1 day of exposure to cold and the subsequent return to control levels as well as the rise in adrenal norepinephrine after 7 days were similar to the changes found after immobilization stress (23).

With chronic exposure to cold, rats become increasingly sensitive to epinephrine (14, 21, 26) and epinephrine (7, 8, 19) and gain weight more rapidly (Table 1). The greater weight of the rats exposed to cold for 42 days may reflect increased fat insulation and a consequent diminution of the stressor effects of exposure to cold. The fall in adrenal TH and DBH levels thus may reflect the decrease in synthesis of catecholamines secondary to the increased tissue sensitivity to these compounds or to a decrease in the effectiveness of cold as a stress in the larger animals.

Thus there are several phases of the response of rat adrenal glands to prolonged cold exposure. At first there is
release of catecholamines, then an increase in the levels of adrenal catecholamine-synthesizing enzyme levels with a return of epinephrine content to normal and finally, as the rats become acclimated to cold by mechanisms considered above, levels of the enzymes decrease.

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