Studies on Adrenaline-Induced Lipolysis in Adrenalectomized Rats

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Synopsis

In a short-term study, adrenaline-induced lipolysis was less in adrenalectomized rats than in controls, though the cyclic AMP accumulation was not different. In adrenalectomized rats treated with corticosterone, lipase activity was as low as in untreated adrenalectomized rats, although adrenaline-induced lipolysis was not reduced. In a long-term study, no reduction in adrenaline-induced lipolysis or cyclic AMP accumulation was observed in adrenalectomized rats. The mechanism of the effect of adrenalectomy on adrenaline-induced lipolysis is discussed on the basis of these results.

The mechanism of adrenaline-induced lipolysis was reported to be closely related to cyclic AMP (Rizack, 1961, 1964; Butcher et al., 1966; Tsai et al., 1970). It was also found that glucocorticoids were required for the normal lipolytic response to adrenaline or ACTH (Jeanrenaud and Renold, 1960; Allen and Beck, 1972; Exton et al., 1972). Various sites have been suggested for the biochemical lesions induced by adrenalectomy. Schönhofer et al. (1966) reported that the lesion was at or before the activation of adenyl cyclase; Corbin and Park (1969) found evidence of tissue insensitivity, and impaired accumulation of cyclic AMP in response to adenyl cyclase activating hormones; it was also reported that phosphodiesterase activity was increased by adrenalectomy (Schönhofer et al., 1972). On the other hand, the mechanism of adrenaline-induced lipolysis other than the cyclic AMP theory was recently reported by Okuda et al. (1974 a and b). The present studies were carried out to investigate the mechanism of the reduction of adrenaline-induced lipolysis in adrenalectomized rats.

Materials and Methods

Animals

Male Sprague-Dawley rats were fed laboratory chow ad libitum. The rats were kept in an animal house at a constant temperature of 23±1°C with exposure to natural light and darkness, and were housed in 4 to one cage. Adrenalectomy was performed by the paralumbar approach under pentobarbital anesthesia. Rats were sacrificed by cervical dislocation and their epididymal adipose tissue was quickly excised.

Chemicals

L-Epinephrine was obtained from Wako Co., Ltd. Dibutyryl cyclic AMP (DBeAMP), collagenase, corticosterone, and bovine serum albumin were obtained from Sigma Co., Ltd. The radioimmunoassay kit was purchased from Schwarzmann Co.. Ediol (coconut oil emulsion) was obtained from Calbiochem Co., Los Angeles.

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Experimental conditions
Two experiments were done, first, 18 hr after adrenalectomy (short-term study) and second, 28 days after adrenalectomy (long-term study). Adrenalectomized rats were given 0.9% NaCl solution instead of water. The following groups were set up:
I) Short-term study (18 hr after adrenalectomy)
   i) control (sham operation)
   ii) control treated with corticosterone (5.0 mg/rat)
   iii) adrenalectomy
   iv) adrenalectomy treated with corticosterone (5.0 mg/rat)
Corticosterone was administered subcutaneously 18 hr before sacrifice. Rats weighed 200 g each.
II) Long-term study (28 days after adrenalectomy)
   i) control
   ii) control treated with corticosterone (0.5 mg/rat/day)
   iii) adrenalectomy
   iv) adrenalectomy treated with corticosterone (0.5 mg/rat/day)
Corticosterone was given subcutaneously every day for the last 14 days before sacrifice (from experimental day 14 to 28). At the beginning of experiments, rats weighed about 150 g.

Preparation of isolated fat cells
Fat cells were isolated by the method of Rodbell (1964).

Estimation of lipolysis
Fat cell suspension, equivalent to 300 mg of adipose tissue, in 3 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% (w/v) bovine serum albumin was incubated with or without a lipolytic agent such as adrenaline or DBCAMP in a final volume of 4 ml. Incubation was performed for 60 min at 37°C under 95% O₂-5% CO₂, and then free fatty acid (FFA) released into the medium was estimated by the method of Itaya and Ui (1956). Lipolytic activity was calculated as the difference between the FFA released in the presence and absence of lipolytic agents.

Estimation of cyclic AMP accumulation in the fat cells
Three ml of fat cell suspension, equivalent to 300 mg of adipose tissue, was incubated in the presence or absence of adrenaline for 10 min at 37°C under 95% O₂-5% CO₂ in a final volume of 4 ml. The incubation was terminated by adding 4 ml of 5% cold trichloroacetic acid (TCA). The mixture was then frozen and thawed 3 times and centrifuged. The resultant supernatant was washed with cold ether to remove TCA and a sample of 0.5 ml was dried at 60°C in a stream of N₂ gas. The residue was dissolved in 0.05 M acetate buffer (pH 6.2), and a sample was used to estimate cyclic AMP. Cyclic AMP was estimated with a radioimmunoassay kit.

Results
Plasma corticosterone level, weight and protein concentration of adipose tissue after adrenalectomy
The plasma corticosterone level was remarkably decreased in adrenalectomized rats, but it increased slightly on administration of corticosterone as shown in Table 1. These results indicate that the adrenals were removed completely and that administration of corticosterone was effective. In adrenalectomized rats the weight and protein concentration of the adipose tissue remained unchanged in the short-term study but decreased greatly in the long-term study. The weight of the adipose tissue in adrenalectomized rats was slightly restored by administration of corticosterone and the protein concentration of the adipose tissue recovered
completely to the level of controls in the long-term study. Triglyceride concentration in fat cells was not significantly different in each group in the short-term study (Table 1).

Adrenaline-induced lipolysis and cyclic AMP accumulation in fat cells in short-term studies

Adrenaline-induced lipolysis was considerably reduced in adrenalectomized rats and remarkably increased in adrenalectomized rats administered with corticosterone.

Table 1. Plasma corticosterone, and weight and protein concentration of adipose tissue after adrenalectomy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Plasma (ng/dl)</th>
<th>Tissue weight (g)</th>
<th>Protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short-term studies</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>18.3 ± 0.95</td>
<td>0.85 ± 0.11</td>
<td>11.05 ± 0.28</td>
</tr>
<tr>
<td>Control + B</td>
<td>200</td>
<td>14.7 ± 1.03</td>
<td>0.85 ± 0.12</td>
<td>11.09 ± 0.22</td>
</tr>
<tr>
<td>Adrex 1)</td>
<td>200</td>
<td>2.1 ± 0.20</td>
<td>0.85 ± 0.21</td>
<td>11.81 ± 0.25</td>
</tr>
<tr>
<td>Adrex + B</td>
<td>200</td>
<td>4.2 ± 0.41</td>
<td>0.85 ± 0.22</td>
<td>11.51 ± 0.29</td>
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<tr>
<td><strong>Long-term studies</strong></td>
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<tr>
<td>Control</td>
<td>326 ± 7.20</td>
<td>20.8 ± 1.40</td>
<td>2.00 ± 0.34</td>
<td>12.96 ± 0.68</td>
</tr>
<tr>
<td>Control + B</td>
<td>320 ± 8.31</td>
<td>27.3 ± 1.86</td>
<td>2.05 ± 0.46</td>
<td>16.68 ± 0.72</td>
</tr>
<tr>
<td>Adrex</td>
<td>284 ± 9.02</td>
<td>2.4 ± 0.26</td>
<td>0.86 ± 0.43</td>
<td>5.69 ± 0.96</td>
</tr>
<tr>
<td>Adrex + B</td>
<td>278 ± 8.30</td>
<td>9.3 ± 2.03</td>
<td>1.35 ± 0.79</td>
<td>19.30 ± 1.03</td>
</tr>
</tbody>
</table>

1) Body weight per rat: mean ± SE of values in 3 experiments.
2) Plasma corticosterone: mean ± SE of values in 10 animals.
3) Epididymal adipose tissue weight (g/rat): mean ± SE of values in 3 experiments.
4) Protein per epididymal adipose tissue weight (mg/g): mean ± SE of values in 3 experiments.
5) Sham operated and corticosterone-treated rats.
6) Adrenalectomized rats.
7) Triglyceride concentration in fat cells in the short-term studies; Control: 59.2 ± 0.9, Control + B: 57.2 ± 1.2, Adrex: 61.2 ± 1.5, and Adrex + B: 58.8 ± 1.8 mg/mg protein (mean ± SE of values in 3 experiments).

Fig. 1. Adrenaline-induced lipolysis and cyclic AMP accumulation on the 2nd day after adrenalectomy. 10⁻⁶ M adrenaline was added to the reaction mixture. Columns and bars are means ± SE of values in 12 experiments.
1) control + corticosterone: sham operated rats administered with corticosterone.
2) adrex: adrenalectomized rats.
3) adrex + corticosterone: adrenalectomized rats administered with corticosterone.
On the other hand, no significant difference was observed in adrenaline-induced cyclic AMP accumulation in controls and adrenalectomized rats. But cyclic AMP accumulation was slightly apt to be decreased in adrenalectomized rats administered with corticosterone than adrenalectomized ones (Fig. 1). DBcAMP-induced lipolysis was reduced in adrenalectomized rats, and was restored to the same level as that in the controls by administration of corticosterone (Fig. 2).

Adrenaline-induced lipase activity in short-term studies

The reductions of adrenaline-induced lipolysis and lipase activity were observed concomitantly in adrenalectomized rats. But lipase activity in adrenalectomized rats administered with corticosterone was as low as in untreated adrenalectomized ones (Fig. 3).

Adrenaline-induced lipolysis, cyclic AMP accumulation and lipase activity in long-term studies

Neither reduction of adrenaline-induced lipolysis nor cyclic AMP accumulation was observed in adrenalectomized rats (Fig. 4). No reduction of DBcAMP-induced lipolysis by adrenalectomy was observed (Fig. 5). Adrenaline-induced lipase activities were
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Discussion

The mechanism of adrenaline-induced lipolysis has been explained in terms of the cyclic AMP theory, in which cyclic AMP plays a regulatory role (Rizack, 1964; Butcher et al., 1966; Tsai et al., 1970). According to this theory, adrenaline stimulates adenyl cyclase in fat cell membranes and increases the level of cyclic AMP. The increased cyclic AMP then stimulates protein kinase activity, which, in turn, activates the hormone-sensitive lipase (Huttunen et al., 1970). If this is true, the reduction of adrenaline-induced lipolysis in adrenalectomized rats may be due to the change in the process beyond cyclic AMP production because cyclic AMP accumulation was not decreased in adrenalectomized rats (Fig. 1). Furthermore, the greater increase in adrenaline-induced lipolysis in adrenalectomized rats administered with corticosterone than in adrenalectomized ones may be due to the change in the process beyond cyclic AMP production because cyclic AMP accumulation was slightly more decreased in adrenalectomized rats administered with corticosterone than in adrenalectomized ones (Fig. 1).

The reduction of DBcAMP-induced lipolysis in adrenalectomized rats and restoration by administration of corticosterone also suggest that the reduction of lipolysis by adrenalectomy is due to the change in the process beyond cyclic AMP production. Therefore, we then examined the correlation between lipase activity and lipolysis.

Lipase activity in adrenalectomized rats administered with corticosterone was as low as in untreated adrenalectomized rats, although adrenaline-induced lipolysis was not reduced in adrenalectomized rats administered with corticosterone (Fig. 3). These results suggest that adrenaline-induced lipolysis might be explained by some mechanism other than the cyclic AMP theory. Recently, it was reported that the stimulation of lipolysis in fat cells is not mediated by activation of lipase activity but by initiation of the reaction between lipase and triglycerides: lipid micelles were prepared from adipose tissue (Okuda et al., 1974) and adrenaline-induced lipolysis was observed without activation of protein kinases.
in the lipid micelles (Saito et al., 1974 b). Furthermore, it was suggested that phospholipid might play a crucial role in adrenaline-induced lipolysis (Saito et al., 1974 a). Therefore, it seems possible that the metabolism of phospholipid, which is the essential factor for the effect of adrenaline, might be changed by adrenalectomy.

In long-term studies, neither reduction of adrenaline-induced lipolysis and cyclic AMP accumulation in adrenalectomized rats, nor reduction of DBcAMP-induced lipolysis by adrenalectomy were observed (Figs. 4, 5). These results suggest that in the long-term studies some factors appear to restore the reduced lipolysis observed in the short-term studies. According to the cyclic AMP theory, these results suggest, therefore, that there are factors in the process beyond cyclic AMP production which restore adrenaline-induced lipolysis. Adrenaline-induced lipase activity was not changed in four experiments in long-term studies (Fig. 6). These results suggest that some factors that restore adrenaline-induced lipolysis, such as factors to elicit the reaction between lipase and triglyceride, might appear within 28 days after adrenalectomy. Further experiments are now in progress on the mechanism of the reduction of adrenaline-induced lipolysis after adrenalectomy.

References

Itaya, K. and M. Ui. (1965). J. Lipid Res. 6, 16.