ADP RIBOSYLATION AND G PROTEIN REGULATION IN THE THYROID

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Although most of the metabolic effects of TSH on the thyroid reflect its activation of the adenylate cyclase-cAMP system (1), other signalling systems mediate the effect of other agonists such as acetylcholine (2) and phorbol esters (3). Furthermore effects of TSH on desensitization (4) and 32P incorporation into phospholipids (1) are not mediated by cAMP. The phosphatidylinositol 4,5-bisphosphate cascade which increases intracellular Ca\(^{2+}\) and activates protein kinase C is present in the thyroid and may be important for the regulation of several metabolic effects (5-9). ADP ribosylation of various proteins is another possible signalling system for cell regulation (10-13). Although this process may involve either poly ADP ribosylation or mono ADP ribosylation, the present discussion will be limited to the latter process.

Vitti et al reported that a 40 KDa protein was ADP ribosylated in bovine thyroid plasma membranes and that such ADP ribosylation was increased by TSH (10). This same substrate was ADP ribosylated by cholera toxin. The effect of TSH was present within 1 minute, was 80% maximal by 2 min and was dependent on the amount of TSH. 1 nM TSH produced an effect which was maximal with 100 nM. Thyroglobulin, insulin, FSH, LH and HCG were ineffective. The TSH stimulation of ADP ribosylation was postulated to be linked to the activation of adenylate cyclase since an inhibitor of the TSH stimulated ADP ribosylation also decreased the hormone's activation of adenylate cyclase. Furthermore TSH stimulation of ADP ribosylation was more rapid and achieved maximal rates faster than its stimulation of adenylate cyclase. Finally when membranes were prepared in hypotonic buffer, NAD increased TSH stimulation of adenylate cyclase.
Filetti and Rapoport also reported that TSH stimulated ADP ribosylation in permeabilized dog thyroid cells as shown in Table 1 (11). The maximum effect was present in 30-60 minutes and required 100 mU/ml hormone. The stimulation involved the ADP ribosyl transferase rather than any change in the substrate pool size or specific activity since it was evident even when a 1000 fold excess of NAD was added. Mono ADP ribosylation rather than poly ADP ribosylation was primarily involved. This effect of TSH was not mediated by cAMP since it was inhibited by dibutyryl cAMP and isobutylmethylxanthine. The stimulation of ADP ribosylation was linked to TSH-induced desensitization (11-13), a process which is independent of cAMP (4) but related to NAD. Thus nicotinamide (50 mM) and N'-methyl nicotinamide inhibited both TSH stimulated ADP ribosylation and desensitization (Table 2). Since N'-methyl nicotinamide cannot be incorporated into an oxidatively functional NAD, the effect of NAD must be other than on electron transport. In addition, nicotinamide accelerated the recovery from TSH-induced desensitization. Arginine and arginine methyl ester which can be ADP ribosylated also inhibited TSH-induced desen­sitization in thyroid 19 HT cells (12). These compounds decreased cholera toxin mediated ADP ribosylation providing further evidence for the hypothesis that mono ADP ribosylation is important in TSH desensitization. However, this effect of arginine and arginine methyl ester could not be obtained in other types of thyroid cells. Other inhibitors of poly ADP ribose polymerase such as thymidine, 5 bromouridine and pyridoxine also prevented TSH desen­sitization. In contrast to TSH-induced desensitization, nicotina­mide did not prevent homologous desensitization due to prostaglandin E1 or adenosine.

### TABLE 1

Effect of TSH on ADP ribosylation in dog thyroid cells

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>0</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol ADP ribose/mg protein</td>
<td>0</td>
<td>0.47 ± 0.08</td>
<td>0.76 ± 0.01</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>0.75 ± 0.05</td>
<td>1.32 ± 0.29</td>
</tr>
<tr>
<td>TSH (100 mU/ml)</td>
<td>-</td>
<td>0.75 ± 0.05</td>
<td>1.32 ± 0.29</td>
</tr>
</tbody>
</table>