infusion for 96 h, toxicity is again very high. LD-50 being more than 50 times lower than after a single injection. Blood and tissue levels can be assumed to run a similar course after epicutaneous administration and during continuous infusion of small doses. This similarity suggests that high toxicity is related to a pharmacokinetic feature, namely long persistence of drug in the blood and/or tissues. This in turn could indicate that some in vivo metabolic process of low capacity transforms ovalicin into a highly toxic compound, and that this process is more active in guinea pigs than in the other animals tested which are less susceptible to the drug. Higher drug doses given p.o., i.v. or i.p. may be eliminated before the slow metabolism has toxified enough of the compound to produce lethality. Apparently, the toxicity of the metabolite is cumulative. A similar situation was observed with the cyclic peptide chlamydocin, which also contains an epoxy group; the LD-50 of this cytostatic compound is 23 times higher when it is injected i.v. as a bolus than when it is infused i.v. over 96 h. Unfortunately, no pharmacokinetic data are available for ovalicin.

The symptoms elicited by cutaneous ovalicin administration suggest that toxicity is mainly due to an effect on the central nervous system. Whether lethality is also brought about by a central action is not clear. Since tranquilizing the drug-treated animals with a neuroleptic (thioridazine) or administration of fluid did not reduce lethality (results not shown), neither the excitation nor the adipsia provoked by ovalicin seems to be the cause of death. The question arises, where metabolic toxification of ovalicin takes place. Is it in the liver? The comparatively high therapeutic index for the immunosuppressive activity of ovalicin suggests that the lymphatic system may be a tissue where toxification takes place. This would be compatible with the high in vivo toxicity of the compound for lymphocytes. P-815 mastocytoma cells, for which in contrast to other cell lines ovalicin is toxic too, may also convert the drug into a toxic metabolite. Is there perhaps even some toxification in parts of the central nervous system, eliciting thus the ovalicin syndrome? Such questions cannot be answered yet, but studies to elucidate these problems may lead not only to a better understanding of the effects of ovalicin but also to new ways to arrive at lymphocyte-specific, immunosuppressive compounds.

Acknowledgment: The skilful technical assistance of Armin Trippmacher and Barbara Burkhardt is gratefully acknowledged.


0014-4754/88/070611-03$1.50 + 0.20/0 © Birkhäuser Verlag Basel 1988

Effect of high doses of somatostatin on adenylate cyclase activity in peripheral mononuclear leukocytes from normal subjects and from acute leukemia patients

M. Peracchi, F. Bamonti-Catena and B. Bareggi

Istituto di Scienze Mediche, Padiglione Granelli, Università di Milano, Via F. Sforza 35, I-20122 Milano (Italy)

Received 10 November 1987; accepted 9 March 1988

Summary. In normal lymphocytes somatostatin non-competitively inhibited basal (IDso 5 x 10^-4 M) and isoproterenol- and forskolin-stimulated adenylate cyclase activity (Ac). In acute leukemia blasts, non-responsive to isoproterenol, forskolin, which activates the catalytic subunit, stimulated and somatostatin inhibited Ac, thus indicating the leukemic enzyme, though defective, retains the inhibitory pathway and catalytic function.

Key words. Adenylate cyclase; somatostatin; forskolin; isoproterenol; lymphocytes; leukemic cells.

Somatostatin, a peptide originally isolated from the hypothalamus, as GH release inhibiting factor, has subsequently been located in many different tissues where it influences a wide variety of cellular processes. Receptors for somatostatin have also been found in human mononuclear leukocytes and evidence is accumulating that this peptide may modulate some lymphocyte functions. In vitro experiments have demonstrated that somatostatin at a concentration of 10^-7 M stimulates lymphocyte proliferation and abolishes the antiproliferative effect of rat hypothalamic extracts, whereas at lower concentrations it clearly inhibits both spontaneous and mitogen-stimulated lymphocyte proliferation, as well as immunoglobulin synthesis induced by concanavalin A. The mechanisms by which somatostatin affects lymphocyte functions are still unknown. In many tissues, however, the effects of the peptide have been related to its ability to inhibit cyclic AMP (CAMP) production. It seemed therefore to be interesting to investigate the possible influence of different doses of somatostatin on adenylate cyclase activity in peripheral mononuclear leukocytes from normal subjects, and in human leukemic leukocytes, which are known to have defective adenylate cyclase unresponsive to various agents. This paper presents evidence that in both normal and leukemic leukocytes somatostatin does not influence adenylate cyclase activity at physiological concentrations, nor at the pharmacological concentrations usually employed, but at higher doses of the peptide enzyme activity is inhibited.
Effect of somatostatin on basal and stimulated adenylate cyclase activity in whole extracts of peripheral mononuclear leukocytes from normal subjects and acute leukemia patients. Data are expressed as mean±SE.

<table>
<thead>
<tr>
<th></th>
<th>Normal lymphocytes</th>
<th>ALL lymphocytes</th>
<th>ANLL lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>5x10^-4 M</em></td>
<td><em>5x10^-5 M</em></td>
<td><em>10^-5 M</em></td>
</tr>
<tr>
<td>Somatostatin</td>
<td>18.5±2.14</td>
<td>3.9±0.61</td>
<td>6.4±2.16</td>
</tr>
<tr>
<td>Control</td>
<td>25.9±2.51</td>
<td>4.3±0.85</td>
<td>6.8±2.10</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>50.0±14.35</td>
<td>32.2±5.13</td>
<td>31.4±7.89</td>
</tr>
<tr>
<td>Forskolin</td>
<td>55.3±11.84</td>
<td>21.4±6.19</td>
<td>19.7±4.75</td>
</tr>
</tbody>
</table>

*p < 0.01 vs control (Student’s t-test); *p < 0.01, ~p < 0.05 vs without somatostatin (two-way ANOVA).

Discussion. The finding that in normal mononuclear leukocytes somatostatin inhibited both basal and stimulated adenylate cyclase activity only at high, unphysiological, concentrations suggests that the modulating influences of the peptide on lymphocyte functions cannot be attributed to a cAMP level decrease, but must be mediated by different mechanisms. These could include actions at steps beyond the catalytic subunit, and transducing system, which are independent and are influenced by different substances. Further pharmacological studies could be interesting, based on the finding that the various components of the adenylate cyclase complex (membrane receptors, catalytic subunit, and transducing system) can be functionally independent and are influenced by different substances.

However, control validity constitutes a major problem in the...