concentration are large as compared with their normal range of values. Errors in the interpretation of these results are potentially important, for example in the followup of patients with the nephrotic syndrome or cirrhosis. We recommend that, when the precise result is of importance, the patient's posture on each occasion should be the same for the 20 min before venepuncture.

We are greatly indebted to our subjects for their patience and support, to Mrs. Joan Merry and Mr. Neil Wyllie for their technical help, and to Professor G. H. Bell for comments on the manuscript.

References

N-Ethylmaleimide Prevents Destruction of Corticotropin (ACTH) in Plasma

William Jubiz and George Nolan

Addition of N-ethylmaleimide, an inhibitor of proteolytic enzymes, to samples for plasma corticotropin determinations prevents destruction of the hormone at room temperature for at least 72 h. A concentration of 125 mg per liter of blood is effective. N-Ethylmaleimide is not so effective in preventing corticotropin degradation in whole blood. Use of the inhibitor should make plasma corticotropin determinations more practical and reliable.

Additional Keyphrases: sample handling · conditions for shipping and storage · hormones

Availability of plasma corticotropin (ACTH) radioimmunoassays has contributed enormously to our understanding of the pituitary-adrenal axis under physiological and pathological conditions (1). However, proper collection and preservation of the plasma samples constitute a major drawback to accuracy. To minimize destruction by proteolytic enzymes, blood specimens must be centrifuged at 4°C and the plasma separated immediately and kept frozen. Small hospitals, clinics, and physicians working in rural communities may lack the facilities for proper processing of the specimens. Moreover, there are obvious difficulties when samples must be shipped elsewhere for assay. A way to obviate these difficulties would greatly facilitate plasma corticotropin radioimmunoassays.

Here, we demonstrate that N-ethylmaleimide, an enzyme inhibitor (2), preserves corticotropin in human plasma for as long as 72 h.

Methods

Blood was collected from five normal subjects (three men and two women, 39-45 and 23-44 years old, respectively) at 0800 hours, after a 3-g oral dose of metyrapone on the previous midnight. From each subject, 10-ml blood samples were drawn into evacuated blood-collection tubes (Vacutainer Tubes) containing 286 USP units of lithium heparin. N-Ethylmaleimide (Eastman Kodak Co., Rochester, N.Y. 14650) dissolved in ethanol, 1.25 mg in 10 µl, had been added to half of the tubes before blood was drawn. Final concentration of the inhibitor was 1.25 mg/10 ml. Sample pairs with or without the N-ethylmaleimide were handled as follows: (a) immediate
centrifugation (3000 x g) at 4°C, separation of the plasma, and storage at -20°C; (b) whole blood was left at room temperature for 1 h and then processed as in a; or (c) whole blood was left at room temperature for 24 h and then processed as in a.

In a second experiment, pairs of plasma samples with or without the added N-ethylmaleimide were left at room temperature for 1, 4, 24, 48, and 72 h. To exclude the possibility of interference by N-ethylmaleimide in the assay, the same final concentration of the inhibitor was added to plasma containing low amounts of corticotropin. All samples in experiments 1 and 2 were analyzed for corticotropin by radioimmunoassay (3). Results were analyzed by paired t-test.

Results

Table 1 shows effect on plasma corticotropin determinations of adding N-ethylmaleimide to whole blood. Corticotropin decreased as a function of time whether or not the inhibitor was present, but the decrease was less at all times in the samples containing N-ethylmaleimide. Differences were statistically significant only at 0 and 1 h. The mean decrease from initial corticotropin values with N-ethylmaleimide was 37% at 1 h and 41% at 24 h.

In contrast, Figure 1 illustrates our results for incubation of plasma samples at room temperature in the absence or presence of N-ethylmaleimide. Plasma corticotropin steadily decreased in the samples containing no inhibitor, but the presence of N-ethylmaleimide prevented degradation of immunoreactive corticotropin for as long as 72 h.

Because corticotropin is degraded to some extent while blood is clotting, results of similar experiments on serum were inconsistent.

Table 1. Effect of N-Ethylmaleimide on Plasma Corticotropin Determinations

<table>
<thead>
<tr>
<th>Condition</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood at room temp for 1 h</td>
<td>180</td>
<td>230</td>
<td>206</td>
<td>218</td>
<td>218</td>
<td>210 ± 19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B</td>
<td>200</td>
<td>274</td>
<td>260</td>
<td>254</td>
<td>230</td>
<td>244 ± 29</td>
<td></td>
</tr>
<tr>
<td>Whole blood at room temp for 24 h</td>
<td>167</td>
<td>104</td>
<td>116</td>
<td>169</td>
<td>159</td>
<td>145 ± 33</td>
<td>NS</td>
</tr>
<tr>
<td>B</td>
<td>204</td>
<td>246</td>
<td>304</td>
<td>198</td>
<td>174</td>
<td>225 ± 51</td>
<td></td>
</tr>
</tbody>
</table>

A, without N-ethylmaleimide; B, with 1.25 mg of N-ethylmaleimide per 10 ml of blood.

N-Ethylmaleimide did not interfere in the assay when added to plasma containing low concentrations of corticotropin; without N-ethylmaleimide the result was 22 ng/liter, with N-ethylmaleimide 25 ng/liter. Higher concentrations of the inhibitor (up to 1.25 g/liter) did not improve the results and a lower concentration (0.6 mg/10 ml) was less effective.

Discussion

As have others (4), we found that corticotropin is more stable in plasma than in whole blood. Although N-ethylmaleimide slowed its degradation in whole blood, an average of 41% became nonreactive within 24 h. On the other hand, destruction of corticotropin in plasma at room temperature is prevented by N-ethylmaleimide for at least 72 h. The mechanism of action of the inhibitor is uncertain, but it possibly simply reflects inactivation of proteolytic enzymes (2). Addition of N-ethylmaleimide to samples for plasma corticotropin assay would obviate the problem of destruction of the hormone during collection and shipping, and thus make plasma corticotropin determination more practical and reliable.

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References

3. ACTH radioimmunoassay Kit, Amersham/Searle, 2636 S Clearbrook Drive, Arlington Heights, Ill. 60005.