EFFECT OF SYNTHETIC HUMAN 1,39-CORTICOTROPHIN ON BLOOD GLUCOSE LEVEL IN MICE

By

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ABSTRACT

The hypoglycaemic action of synthetic human 1,39-corticotrophin was determined in mice and its effectiveness compared with highly purified porcine corticotrophin. Synthetic human 1,39-corticotrophin (0.01 mg = 1.0 IU) and porcine corticotrophin (1.0 IU) induced a transient hypoglycaemia. After the administration of the porcine corticotrophin the decrease in the blood glucose concentration was somewhat more marked and prolonged. Adrenocortical activity was not necessary for the development of hypoglycaemia. In adrenalectomized mice, dexamethasone substitution by increasing the initial blood glucose content, made the effect more pronounced. Pretreatment with corticotrophin reduced the extent of the alloxan-induced transitory hyperglycaemia. On the other hand, it did not influence the high blood glucose values in manifest alloxan diabetic animals.

It is possible that corticotrophin induces hypoglycaemia through insulin release. This is an extra-adrenal effect of corticotrophin as it is also observed in adrenalectomized mice.

Lee et al. (1961) first described the chemical structure of human corticotrophin. This polypeptide consisting of 39 amino acids was synthetized by Bajusz & Medzihradszky (1967) and Bajusz et al. (1967). According to previous investigations (Kovács et al. 1968; László et al., in press) the hormone markedly stimulates adrenocortical function. It could, however, be established that the polypeptide consisting of 10 amino-acids already possesses a mild corticotrophic effect (Ney et al. 1965); corticotrophin fragments composed of 23 and 24 amino acids significantly increase adrenocortical function (Danowski et al.).
The effect of corticotrophin is not limited exclusively to the adrenals; it also acts on various extra-adrenal receptor sites (Engel & Lebovitz 1965; Lebovitz 1965). In short term experiments several investigators observed that it causes hypoglycaemia (Engel & Engel 1954; Engel et al. 1958; Lebovitz & Pooler 1967a,b). The decrease in blood sugar also occurs in adrenalectomized rodents (Westermeyer & Raben 1954); however, other investigators found that the presence of cortisone is also necessary for this effect (Love et al. 1965; Lebovitz & Pooler 1967a,b; Lundquist & Rerup 1967).

In the present work the following three questions were studied:

1. Does the blood sugar transitorily decrease following the administration of synthetic human 1,39-corticotrophin?
2. Is the corticotrophin hypoglycaemia prevented by adrenalectomy?
3. How does corticotrophin influence the changes in blood glucose levels induced by alloxan?

**METHOD**

The experiments were performed on 639 non-fasting adult male albino mice weighing 20–30 g kept on a standard diet. Bilateral adrenalectomy was carried out through a lumbar approach under ethyl ether anaesthesia. After the operation 0.9% NaCl solution was given for drinking. The examinations were performed on the first and fourth day after the removal of the adrenals. Some of the mice were pretreated with 0.2 mg dexamethasone (Oradexone®, Organon) given intravenously into the tail vein, 60 min before the blood glucose determination. Corticotrophin was also injected into the tail vein. The biological activity of human 1,39-corticotrophin was determined by the bioassay method of Lipscomb & Nelson (1962) as described by Szporny et al. (1968), the activity being 107 IU/mg. A highly purified porcine corticotrophin (Cortrophine®, Organon) was used in parallel. For comparison the hormone doses were expressed in international units. Alloxan-monohydrate (British Drug Houses Ltd.) was also administered intravenously (85 mg/kg body weight). Blood glucose from the carotid blood was determined at different periods after the corticotrophin injection by the orthotoluidine method.

**RESULTS**

In the first experiment, the effects of 1.0 IU of synthetic human corticotrophin and porcine corticotrophin were studied on the blood glucose levels. The animals were killed at different period after the intravenous injection of this high dose and the blood glucose concentration was determined. The results are presented in Fig. 1. It can be seen that following the administration of both compounds the blood glucose level decreases. The hypoglycaemia is most pronounced 30 min after treatment. Both the synthetic human as well as the
Fig. 1.
Effect of 1.0 IU synthetic human-, and 1.0 IU highly purified porcine corticotrophin on the blood glucose level in mice (as a function of time). 10 animals in each group. Abscissa: time in minutes after intravenous corticotrophin injection. Ordinate: blood glucose level, mg/100 ml. Vertical bars indicate standard error of mean.

Porcine corticotrophin induced a reduction in the blood glucose content of about 50%. The effect of purified porcine corticotrophin starts earlier, lasts longer, and is more protracted, but after 90 min both curves approach the initial values.

In the following experiments the mice were always exsanguinated 30 min after the administration of corticotrophin. Various doses of corticotrophin were used. In Fig. 2 it can be seen that small amounts (0.1 mU) of the hormone are already effective and that the blood glucose level decreases approximately linearly with the doses given. Fig. 2 also shows that the two curves run almost parallel, though the porcine corticotrophin appears to be somewhat more active.

Subsequently the problem of whether adrenalectomy prevents hypoglycaemia was examined. The role of glucocorticoids in the development of hypoglycaemia was also analysed. In the first group investigations were performed on the first postoperative day. The animals were always killed 30 min after corticotrophin administration.

The results are summarized in Table 1. It can be seen that the removal of the adrenals *per se* resulted in a 25 mg/100 ml decrease in the blood glucose level, that after corticotrophin injection the blood glucose concentration showed
Fig. 2.
Effect of synthetic human-, and highly purified porcine corticotrophin on the blood glucose level in mice (as a function of the dose). 10 animals in each group. Abscissa: dose of corticotrophin (mU). Ordinate: blood glucose level, mg/100 ml. Vertical bars indicate standard error of mean.

a further reduction and that following the porcine corticotrophin injection, hypoglycaemia was more pronounced. Administration of dexamethasone slightly increased the blood glucose content of mice adrenalectomized one day previously. Corticotrophin also caused hypoglycaemia in such pretreated animals.

The majority of the animals drinking isotonic sodium chloride solution died on the fifth and sixth days after the removal of the adrenals, providing glucocorticoid substitution was not given. Therefore, the other examinations were performed on mice adrenalectomized four days previously. The second part of Table 1 demonstrates that as a result of adrenalectomy the blood glucose level of the animals decreased to 57 mg/100 ml, and that further considerable reduction does not follow after the administration of any of the corticotrophins. Dexamethasone significantly increased the glucose content of the blood. If synthetic human or porcine corticotrophin was administered to such pretreated mice the blood glucose level decreased considerably. Statistically there is also a significant difference between these values and those of the dexamethasone treated adrenalectomized mice.

The alloxan-induced acute changes in the blood glucose level are presented in Fig. 3. In this experiment 2.0 IU of corticotrophin was injected intravenously into the tail vein of the animals 10 min before the administration of alloxan-monohydrate. The control group instead of the hormone, was given
Table 1.
Effect of synthetic human and highly purified porcine corticotrophin on blood glucose level in adrenalectomized mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after operation (day)</th>
<th>Treatment</th>
<th>Number of animals</th>
<th>Blood glucose (mg/100 ml)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Non operated control</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>110 ± 1.8*</td>
<td>1/2 P &lt; 0.001</td>
</tr>
<tr>
<td>2. Adrenalectomized</td>
<td>1</td>
<td>-</td>
<td>12</td>
<td>85 ± 3.5</td>
<td>1/8 P &lt; 0.001</td>
</tr>
<tr>
<td>3. Adrenalectomized</td>
<td>1</td>
<td>1.0 IU human ACTH</td>
<td>10</td>
<td>63 ± 1.5</td>
<td>2/3 P &lt; 0.001</td>
</tr>
<tr>
<td>4. Adrenalectomized</td>
<td>1</td>
<td>1.0 IU porcine ACTH</td>
<td>10</td>
<td>46 ± 2.6</td>
<td>2/4 P &lt; 0.001</td>
</tr>
<tr>
<td>5. Adrenalectomized</td>
<td>1</td>
<td>0.2 mg dexamethasone</td>
<td>10</td>
<td>94 ± 4.4</td>
<td>2/5 P &gt; 0.05</td>
</tr>
<tr>
<td>6. Adrenalectomized</td>
<td>1</td>
<td>0.2 mg dexamethasone</td>
<td>12</td>
<td>52 ± 4.1</td>
<td>2/8 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 1.0 IU human ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Adrenalectomized</td>
<td>1</td>
<td>0.2 mg dexamethasone</td>
<td>12</td>
<td>53 ± 3.8</td>
<td>5/6 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 1.0 IU porcine ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Adrenalectomized</td>
<td>4</td>
<td>-</td>
<td>10</td>
<td>57 ± 5.0</td>
<td>5/7 P &lt; 0.001</td>
</tr>
<tr>
<td>9. Adrenalectomized</td>
<td>4</td>
<td>1.0 IU human ACTH</td>
<td>11</td>
<td>49 ± 6.5</td>
<td>8/9 P &gt; 0.05</td>
</tr>
<tr>
<td>10. Adrenalectomized</td>
<td>4</td>
<td>1.0 IU porcine ACTH</td>
<td>11</td>
<td>48 ± 4.3</td>
<td>8/10 P &gt; 0.05</td>
</tr>
<tr>
<td>11. Adrenalectomized</td>
<td>4</td>
<td>0.2 mg dexamethasone</td>
<td>10</td>
<td>84 ± 5.2</td>
<td>8/11 P &lt; 0.01</td>
</tr>
<tr>
<td>12. Adrenalectomized</td>
<td>4</td>
<td>0.2 mg dexamethasone</td>
<td>10</td>
<td>59 ± 3.7</td>
<td>11/12 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 1.0 IU human ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Adrenalectomized</td>
<td>4</td>
<td>0.2 mg dexamethasone</td>
<td>10</td>
<td>58 ± 3.1</td>
<td>11/13 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 1.0 IU porcine ACTH</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Standard error.
Fig. 3.
Effect of 2.0 IU synthetic human-, and 2.0 IU highly purified porcine corticotrophin on the blood glucose changes induced by alloxan. 20 animals in each group. Abscissa: time in minutes after intravenous alloxan administration (85 mg/kg body weight). Ordinate: blood glucose level, mg/100 ml. Vertical bars indicate standard error of mean.

a solution of isotonic sodium chloride. Blood glucose was determined 45 and 360 min following alloxan administration. The control group showed the characteristic curve; following a transitory hyperglycaemia, the blood glucose level fell below the normal. Pretreatment with corticotrophin preparations significantly diminished the acute rise in blood glucose and slightly antagonized the hypoglycaemia developing 6 h later.

The last series of investigations were performed on mice with manifest diabetes on the third day after alloxan treatment (Fig. 4). The initial blood glucose values of the animals were very high and this could not be influenced by the corticotrophin preparations; in the mice with permanent alloxan diabetes the hypoglycaemia inducing effect was not apparent.

DISCUSSION
A decrease in the blood level was first demonstrated by injecting a crude pituitary extract (Harsters 1933; Hoffmann & Anselmino 1933; Westermeyer & Raben 1954); later the phenomenon was reproduced with purified animal
corticotrophin preparations (Engel & Engel 1954; Engel et al. 1958). After the synthesis of various polypeptides with a stimulating action on the adrenal cortex, interest was directed towards the synthetic corticotrophin fragments.

Transitory extra-adrenal hypoglycaemia has been described following the administration of compounds containing 16-, 20- and 24-amino acids (Lebovitz & Engel 1964; Genuth & Lebovitz 1965; Lebovitz 1965; Lundquist & Rerup 1967). Our experiments prove that the administration of synthetic complete human corticotrophin to mice also elicits the phenomenon. Although both the purified porcine corticotrophin, and the synthetic hormone significantly reduces the blood glucose levels, there are some minor differences between the effects of these two compounds.

There is some evidence that corticotrophin preparations do not exert their blood sugar reducing effect through the adrenals (Engel & Engel 1954; Engel et al. 1958; Engel & Lebovitz 1965; Lebovitz 1965; Lebovitz & Pooler 1967a). It was found that corticotrophin causes a transitory hypoglycaemia in adrenalectomized mice kept on a NaCl solution (Westermeyer & Raben 1954). Recent experiments, however, showed that the presence of glucocorticoids is necessary for the development of such a corticotrophin effect (Love et al. 1965; Lebovitz &
Pooler 1967b; Lundquist & Rerup 1967). It seems that following adrenalectomy the blood glucose level of the rodents gradually decreases to such an extent that corticotrophin cannot lower it any more. This assumption is supported by the observations of Genuth & Lebovitz (1965) who found that in adrenalectomized animals, corticotrophin administration in spite of increasing blood insulin level does not markedly alter the blood glucose concentration.

Our results demonstrate that the 1 and 4 days previously operated groups do not react in a similar way to corticotrophin. Without dexamethasone-treatment, the administration of corticotrophin does not further decrease the blood glucose level in mice which is already reduced 4 days following adrenalectomy. Lundquist & Rerup (1967), however, assume that the initial low blood glucose level of the adrenalectomized animals does not play an important role in the failure of the development of this phenomenon.

Some data suggest that in rodents, corticotrophin is capable of releasing insulin, which would explain the reduction in blood glucose (Westermeyer & Raben 1954; Engel et al. 1958; Genuth & Lebovitz 1964, 1965; Love et al. 1965; Lebovitz et al. 1965; Lebovitz & Pooler 1967a; Lundquist & Rerup 1967). We did not perform any insulin determinations. However, the fact that corticotrophin does not reduce the elevated blood glucose levels in alloxan diabetic mice, also suggests that insulin release is involved in the hypoglycaemic effect. Lundquist & Rerup (1967) made similar observations using a corticotrophin fragment containing 24 amino acids.

REFERENCES


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